

Ph.D. Thesis

Abiotic synthesis of organic matter in aqueous
environments simulating parent bodies of
meteorites and the effects of minerals on the
production of amino acids

隕石母天体を模擬した熱水環境における有機物の非生物
的合成とアミノ酸の生成に対する鉱物の影響

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Abstract

The early Solar System comprised a broad area of abiotically created organic compounds, including interstellar organics which were integrated into planetesimals. Some of these planetesimals were incorporated into planets, while their remnants were found in the form of asteroids and comets. Most of the meteorites are came from asteroids that broke up into pieces, which means that some asteroids can be the parent bodies of meteorites. Eventually, the organic compounds were delivered to the early Earth through meteorites. Indeed, some meteorites called “carbonaceous chondrites” are known to contain abiotically synthesized amino acids and other organic compounds. I aimed to evaluate the stability of simulated interstellar complex organic compounds under high temperature and pressure conditions simulating parent bodies of meteorites. Water ice was accreted along with anhydrous minerals by most chondrite parent bodies and thereafter underwent aqueous/hydrothermal alteration when the ice melted in response to heating, mostly from radioactive decay of ^{26}Al .

I synthesized simulated interstellar complex organic compounds by proton irradiation of a gas mixture of carbon monoxide, ammonia, and water, as an analog of complex amino acid precursors, which are known to release amino acids, predominantly glycine (Gly) and alanine (Ala) after acid hydrolysis, on the basis of Kobayashi et al. (1995) who reported that at the first stage of chemical evolution, the main compounds formed abiotically are complex organic compounds with high molecular weights. Therefore, I examined their possible hydrothermal alteration and stabilities as amino acid precursors under high temperature (200–300 °C) and pressure (8–14 MPa) conditions simulating parent bodies of meteorites by using an autoclave. The resulted products were divided into two aliquots; the first one was subjected to UV absorption and UV fluorescence spectroscopy, while the other was acid hydrolyzed and was subjected to amino acid analysis after acid hydrolysis, using high-performance liquid chromatography (HPLC).

All samples treated at 200–300 °C predominantly released glycine and alanine. After heating, amino acid concentrations decreased with increasing temperature in general, but approximately 40% of glycine and alanine could survive at 200 °C. However, the recovery ratios of γ -aminobutyric acid (γ -ABA) increased after heating, indicating that γ -ABA was synthesized during hydrothermal alteration.

The interstellar complex organic analog could maintain as amino acid precursors after being treated at high temperature and pressure. However, their molecular structures were

altered during heating to form organic compounds that are more stable and can survive in elevated hydrothermal conditions in meteorite parent bodies.

In addition to the amino acid precursor formations in interstellar environments, it is known that the amino acids could have been synthesized in the meteorite parent bodies during hydrothermal alteration. Minerals could play an important role in the evolution of meteoritic organic matter, but the effects of minerals on the synthesis of organic matter in parent bodies are not well known. Thus, I aimed to evaluate the role of minerals on the amino acids production in environments simulating hydrothermal alteration in parent bodies of meteorites.

Hydrothermal experiments were conducted using a mixture of formaldehyde and ammonia that are ubiquitously found in interstellar and in the Solar System, with the presence of water simulating aqueous alteration in meteorite parent bodies. Three kinds of minerals were added which are typical in meteorites one by one to the previous mixture to evaluate their catalyzing/inhibiting impact on amino acids production at 150°C over reaction periods of 1 d, 3 d, or 7 d. The resulted products were analyzed using Fourier transform infrared (FTIR) microspectroscopy and gel filtration chromatography, and amino acid analysis using HPLC.

The aqueous solution of formaldehyde and ammonia produced various kinds of amino acids after acid hydrolysis. Minerals showed catalytic effects for the formation of amino acids at shorter heating duration (1 d), especially with montmorillonite. Olivine and serpentine enhanced amino acids production for 1 d and 3 d. After 7 d, all minerals enhanced amino acid decomposition. Consequently, I suggested that minerals enhance the amino acid production at short heating duration, but they turned out to enhance their decomposition for longer heating. Molecular weight distribution in the whole products showed that minerals enhanced both decomposition and combination of molecules. IR spectra revealed that the ester C=O/C=C peak intensity ratios with minerals showed a lower value at short heating duration, maybe due to the enhancement of decarboxylation by minerals, while this ratio increased after heating for 7 d, maybe due to catalytic esterification by minerals.

My results indicated that minerals impacted the formation of amino acids in aqueous environments in meteorite parent bodies, and the amino acids could have different response behaviors according to different minerals. I suggested that the concentrations of the amino acids depend on 1) the presence and the kind of minerals and 2) the stability of the amino acid during the longer heating duration.

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List of publications

Journal article

1. **Elmasry W**, Kebukawa Y, Kobayashi K (2021) Synthesis of Organic Matter in Aqueous Environments Simulating Small Bodies in the Solar System and the Effects of Minerals on Amino Acid Formation. *Life* 11:32. <https://doi.org/10.3390/life11010032>
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3. **Elmasry W**, Kebukawa Y, Shibuya T, Makabe A, Kobayashi K. Thermodynamic model and its experimental correlation for the effect of minerals on amino acid formations in hydrothermal environments simulating terrestrial and extraterrestrial water worlds. “In preparation”

Conferences

1. Elmasry W, Kebukawa Y, Shibuya T, Kobayashi K. Abiotic synthesis of organic matter in aqueous environments simulating parent bodies of meteorites and the effects of minerals on the production of amino acids, in Lunar and Planetary Science Conference (LPSC 52), 15 - 19 Mar. 2021.
2. **Elmasry W**, Kebukawa Y, Kobayashi K. Minerals and their catalytic effects on amino acid formations in environments simulating asteroids, presented at the Japan Geoscience Union, Chiba, Mar. 2020.
3. **Elmasry W**, Kebukawa Y, Kobayashi K. Mineral surfaces and their catalytic effects on amino acid formation during hydrothermal alteration in environments simulating meteorite parent asteroids, The 1st International Workshop for Aquaplanetology, Tokyo Institute of Technology, Tokyo, Mar. 2019.
4. **Elmasry W**, Kebukawa Y, Kobayashi K. Abiotic synthesis of organic matter in environments simulating asteroids and the catalytic effect of minerals on amino acid formation, Japan Geoscience Union, Chiba, Mar. 2019.
5. **Elmasry W**, Misawa S, Matsukuma J, Kebukawa Y, Kobayashi K. Effect of Minerals on Amino Acid Formation in Environments Simulating Parent Bodies of Meteorites, Japan Geoscience Union, Chiba, May. 2018.

6. Misawa S, Kebukawa Y, **Elmasry W**, Yoda I, Muramatsu K, Hamanaka S, Kobayashi K. Formation of amino acid precursors by gamma rays on planetesimals in the early solar system, The 78th Annual Meeting of the Japan Society for Analytical Chemistry, H2002, Yamaguchi university Tokiwa campus, Ube city, Yamaguchi prefecture, May. 2018.
7. **Elmasry W**, Kawamoto Y, Kaneko T, Obayashi Y, Kanda K, Kobayashi K. 21 Alteration of Organic Compounds in Simulated Primitive Solar System Environments, General Session, The 39th Annual Meeting of the SSOEL-Japan Program, 2014.
8. Kobayashi K, Kawamoto Y, **Elmasry W**, Eto M, Tokimura H, Kaneko T, Obayashi Y, Mita H, Kanda K, Yoshida S, et al. Evolution of Interstellar Organics to Meteoritic and Cometary Organics: Approaches by Laboratory Simulations. LPI Contributions 1016, 2013.

Chapter 1

GENERAL INTRODUCTION

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1.1. The solar system formation and the origin of life

Our solar system has been started approximately 4.56 billion years ago when a molecular cloud of matter collapsed under the pressure of gravity. That collapse was probably triggered by a massive supernova explosion, which seeded this cloud of new materials from other supernovae and dying stars. As the cloud collapsed, it began to spin, and it slowly flattened out to form a protoplanetary disk. As the protoplanetary disk began to collapse, at its center, it got hotter, until eventually, fusion began, resulting in the birth of the sun. The majority of the material in the protoplanetary disk went into the sun, while the tiny residues formed the entire solar system. The intense heat of the young sun drove away a lot of hydrogen and helium from the inner parts of the solar system, which gathered condensed to form the outer gassy planets, Jupiter, Saturn, Uranus, and Neptune. The inner rocky planets, including our Earth, have been formed from tiny solid residues orbiting in the inner orbits that eventually gather through collisions to form little rocks. The molecular cloud contained stardust, organic matter, ices, and gas-phase molecules. “Stardust” was a small fraction of the dust in the interstellar medium, consisting of interstellar refractory minerals. Carbonaceous chondrites, which include the most primitive meteorites, have stored those stardust grains prior to the formation of our solar system. A wide range of abiotically created organic compounds in the early solar system, including interstellar organics, were integrated into planetesimals. Some of these planetesimals were incorporated into planets, while their remnants were found in the form of asteroids and comets. Most of the meteorites are came from asteroids that broke up into pieces, which means that some asteroids can be the parent bodies of meteorites. Eventually, the organic compounds were delivered through meteorites and micrometeorites to the early Earth, in which early life probably had started about 3.8 billion years ago (Mojzsis et al., 1996).

1.2. Sources of organic matter in early Earth

The study of the origin of life, besides the evolution of the Earth’s environments, helps us realize why the Earth became suitable to live in and why terrestrial life has continued for billions of years. Endogenous synthesis and exogenous delivery of organic compounds (Fig. 1-1) are suggested to be two sources of the chemical evolution of the early solar system, and organic compounds delivered by either source are considered necessary for the origin of life.

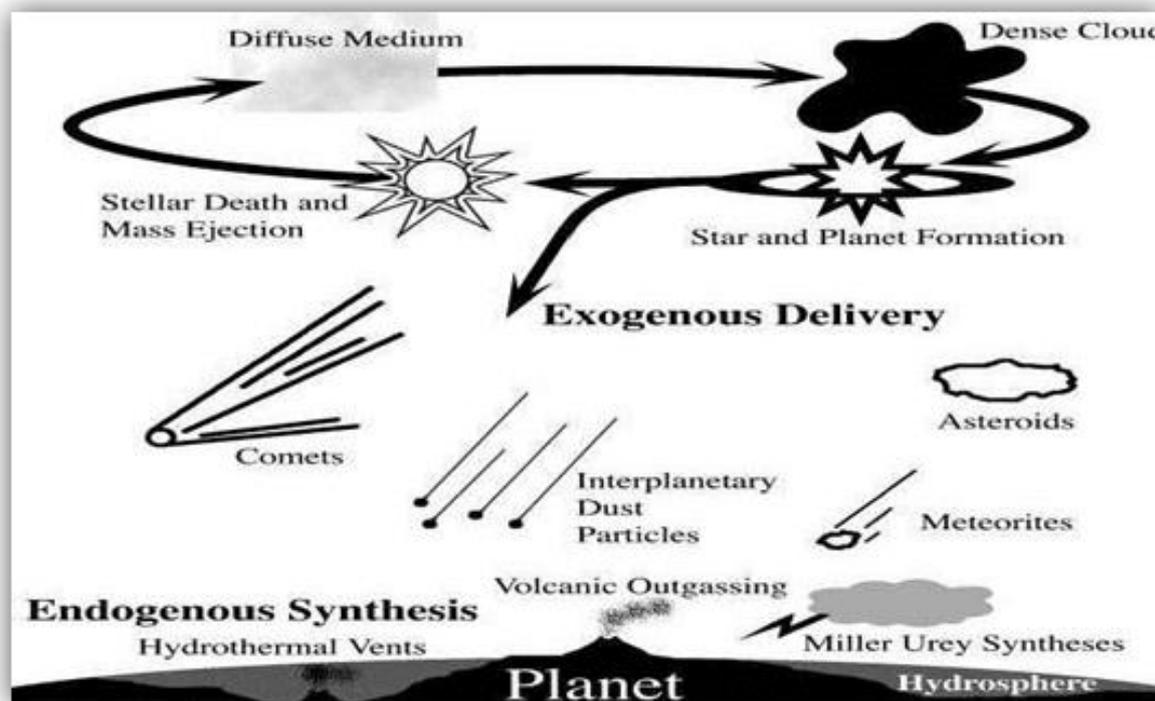


Fig. 1-1 Sources of chemical evolution in the solar system, which was created by Jason Dworkin when he was working at NASA (Dworkin et al. 2001).

1.2.1. Endogenous supply of organic compounds

Many experiments have been conducted, including the historic spark discharge experiment, the Miller-Urey experiment (Miller 1953; Miller and Urey 1959), to reconstruct the origin of life on Earth. They used water (H_2O), methane (CH_4), ammonia (NH_3) and hydrogen (H_2), which were thought the major components of the early Earth's atmosphere. After one week, they noticed that ~10-15 % of the carbon within the system was in the form of organic compounds. Amino acids comprised 2 % of the carbon, with glycine as the most abundant. They proved that under highly reduced conditions, various organic compounds, including amino acids, could be obtained by spark discharges. Other investigators showed that bioorganic compounds could be synthesized from strongly reducing starting mixtures using heat (Harada and Fox 1964), UV radiation (Sagan and Khare 1971; Bar-Nun and Hartman 1978), shock waves (Bar-Nun et al. 1970), and laser-induced plasma energy (Scattergood 1989). Moreover, volcanic outgassing and hydrothermal vents provide alternative sites for organic synthesis (Yanagawa and Kobayashi 1989). On the other hand, it was suggested that they were minor contributors for organic synthesis, for example, submarine hydrothermal vents do not seem to presently synthesize organic compounds more complex than simple hydrocarbons such as CH_4 and ethane (McCollom et al. 2010). It was also claimed that they decompose them over short

time spans ranging from seconds to a few hours (Cleaves 2012). Prebiotic formation of bioorganics in hydrothermal vents is still controversial.

However, the predominant view in the last several years has been that the primitive Earth atmosphere was only “weakly reducing”, composed of a mixture of carbon dioxide, nitrogen, and water, coupled with small quantities of carbon monoxide and hydrogen (Walker 1977; Kasting and Brown 1998). Given this, the Miller-Urey-type synthesis in the primitive Earth atmosphere would have been only a minor contributor to the formation of prebiotic organic compounds (Stribling and Miller 1987). Kobayashi and coworkers showed that amino acid precursors could be synthesized from weakly reducing gas mixtures (e.g., carbon dioxide, carbon monoxide, nitrogen, and water) by irradiation with high-energy protons (Kobayashi et al. 1998; 1999). Thus, endogenous production of organic compounds in a weakly reducing atmosphere could be possible by the action of cosmic rays, but their production rate might have been limited.

1.2.2. Extraterrestrial delivery of organic compounds

Extraterrestrial delivery of organic carbon including amino acids to the early Earth prior to the generation of life through comets, meteorites, and interplanetary dust particles may have been important for the origin of life (Oró 1961; Anders 1989; Chyba and Sagan 1992; Pizzarello and Shock 2010).

1.3. Meteorites

Meteorites are pieces of other solar system bodies which mostly originated from planetesimals or parent asteroids that accreted during the solar system formation (McSween and Huss 2010). In a few cases, they have come from the moon and the planet Mars. Carbonaceous chondrites are a primitive subgroup of stony meteorites which mainly composed of silicates. They are the most primitive known meteorites, and this is why they are considered as extraterrestrial samples of the solar system which preserve records and signatures of prebiotic chemistry and can offer clues about the conditions present during the early solar system. Some carbonaceous chondrites are aqueously altered by water, while others are thermally metamorphosed by heat on their parent asteroids, however, they still carry historical records of their chemical evolution in the universe.

1.4. Organic matter in meteorites

Some carbonaceous chondrites contain up to 3–5 wt % carbon, which is mostly present as higher concentrations and varieties of organic molecules (Pearson et al. 2006; Alexander et al. 2007). The organic matter preserved in carbonaceous chondrites are important tool to understand processes in the outer solar nebula and in the cold molecular cloud. The organic compounds in carbonaceous chondrites were discovered at the beginning in the Alais (CI1) carbonaceous chondrite (Berzelius 1834), which fell in France in 1806. Since then, the organic compounds have been studied. The research studies have been improved in 1969 after the Murchison meteorite fell in Australia. It is considered one of the most important meteorites because it contains a huge number of organic compounds (Sephton 2002). They probably preserved important information about the origin of life in early Earth since it may have been synthesized in the interstellar medium and the solar system.

The early Solar System was comprised of a wide array of organic compounds that were abiotically formed, including interstellar organics that were integrated into planetesimals and the parent bodies of meteorites and then eventually delivered to the early Earth. These organic compounds are divided into soluble organic matter (SOM) and insoluble organic matter (IOM) (e.g., Botta and Bada 2002). The complex insoluble macromolecular organic matter represents the majority of the organic compounds in meteorites (more than 70%), which is insoluble in water or organic solvent. While, the low molecular weight materials represent less than 25% and are soluble in water and organic solvent, which include a wide range of compounds such as carboxylic acids, aliphatic and aromatic hydrocarbons, and amino acids. (Sephton, 2002; Pizzarello et al. 2006). The most abundant class of compounds in the soluble fraction of meteorites are carboxylic acids at approximately 300 parts per million (ppm), followed by more than eighty different amino acids at approximately 60 ppm total quantity, as measured in the CM2 carbonaceous chondrite Murchison (Sephton 2002). The soluble organic content of carbonaceous chondrites provides a unique chemical record of the prebiotic chemical evolution that occurred before and during the formation of the early solar system and subsequent parent body alteration. The origin of these organic compounds is of interest for understanding the origin of life on Earth, as some of these compounds could be precursors to biological molecules (Botta and Bada 2002; Sephton 2002; Glavin et al. 2018).

1.5. Murchison Meteorite

The Murchison meteorite is the most well-studied carbonaceous chondrite for organic matter which is classified as insoluble macromolecular materials (~70%) and simpler soluble compounds (~30%) such as amino acids and carboxylic acids (e.g., Pizzarello et al. 2006). The most abundant soluble compounds (Fig 1-2, Remusat 2014) are carboxylic acids, amino acids, sugar derivatives, and nucleobases. Over one hundred amino acids including α -, β -, γ -, δ -, and ϵ -amino acids were contained in their extracts, and eight of these amino acids are also found in proteins, namely glycine (Gly), alanine (Ala), proline, valine, leucine, isoleucine, aspartic acid (Asp), and glutamic acid, in addition to numerous other compounds encountered in terrestrial metabolisms (Pizzarello and Shock 2010, and references there in). Other compounds also have been detected: alcohols, aldehydes, ketones, and aromatic and aliphatic hydrocarbons. Most classes of these soluble compounds share several characteristics of comprehensive structural diversity and isomerism, exponential abundance decrease within the homologous series, and the branched chain isomers are abundant (Pizzarello 2006).

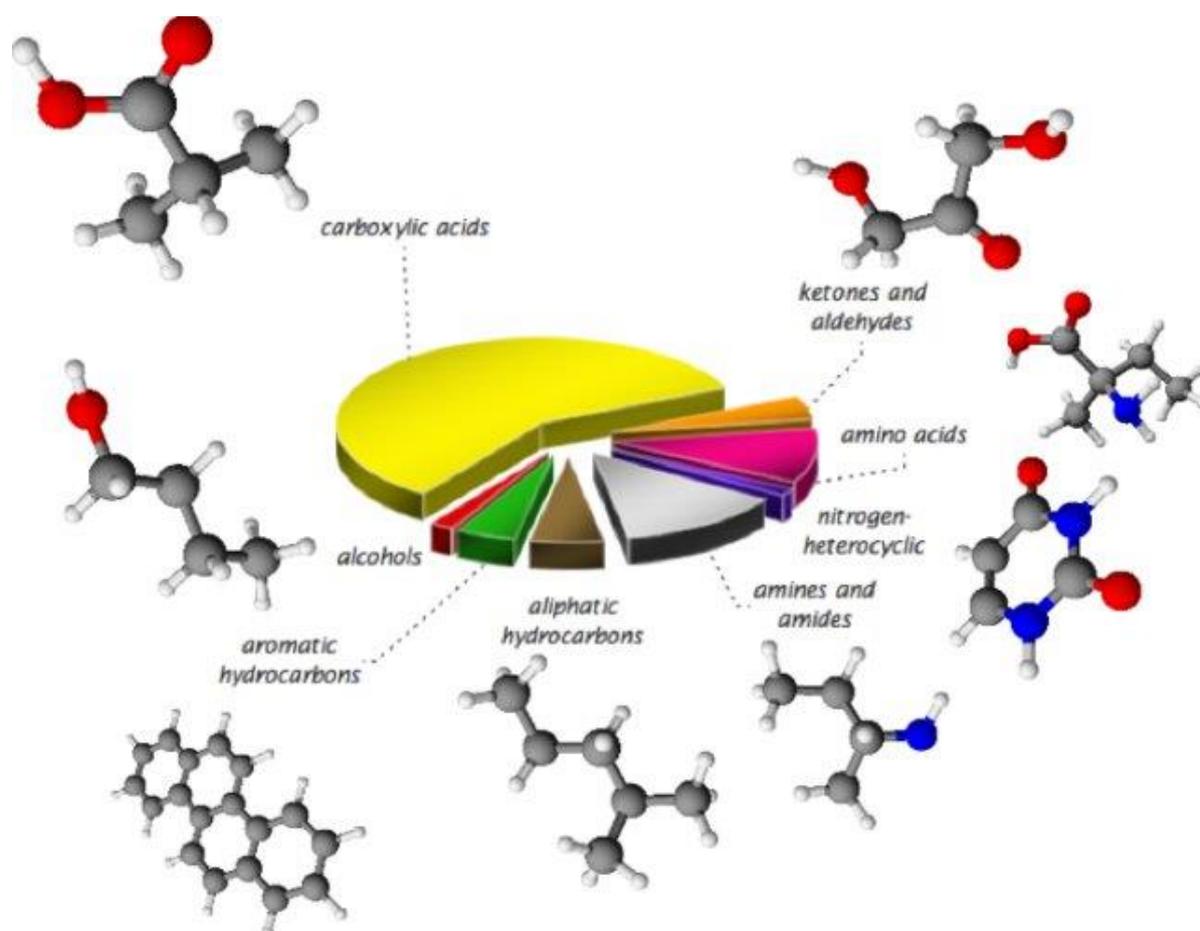


Fig. 1-2 Variety of soluble organics in the Murchison carbonaceous chondrite (Remusat 2014)

1.6. Amino acids formation and sources of energy

Many experiments have been conducted with energy sources in extraterrestrial environments to synthesize hydrocarbons, amino acids, and nucleic acid bases from simple molecules. Ultraviolet irradiation of dust grains in the interstellar medium could be possible energy for complex organic molecule formation. Muñoz Caro et al. (2002) performed a simulation of interstellar ices mixtures of water, carbon dioxide, methanol, and ammonia, placed under a pressure of 10⁻⁷ mbar at 12 K and irradiated with electromagnetic radiation, as well as their analysis by gas chromatography/mass spectrometry. They identified sixteen amino acids, six of which were protein amino acids, in the simulated ice mantle of interstellar dust particles. Moreover, hydantoin, 2,4-imidazolidinedione were formed from simpler ices containing water, methanol, and ammonia via vacuum UV photoirradiation (De Marcellus et al. 2011). The dominant amino acids were formed when 5-substituted hydantoins were UV irradiated and hydrolyzed were Gly and Ala (Sarker et al. 2013). Kobayashi and coworkers simulated interstellar ice by using a starting material composed of a mixture of methanol, ammonia, and water (molar ratio = 1:1:2.8) and irradiated by heavy ions, and they hydrolyzed amino acids by using an ion-exchange high-performance liquid chromatography (IE-HPLC). A wide range of amino acids was detected after acid hydrolysis, including protein amino acids such as Ala, Gly, and Asp as well as non-protein amino acids such as β -alanine (β -Ala) and α - and γ -aminobutyric acid (Kobayashi et al. 2012). Moreover, Complex organic compounds could be synthesized by proton irradiation of a gas mixture of CO, NH₃, and H₂O simulating the interstellar complex organic compounds that are known to release amino acids after acid hydrolysis (Kobayashi et al. 1999; Takano et al. 2004).

1.7. Aqueous / Hydrothermal Alteration

Another possible scenario of the formation of organic matter is via aqueous alteration or a hydrothermal reaction in the meteorite parent bodies. A possible reaction for the formation of α -amino acids is generally considered to be a Strecker-like reaction from precursor aldehydes, ketones, ammonia, and HCN in the presence of water. On the other hand, it has been suggested that chondritic organic matter was produced through the reaction of interstellar formaldehyde followed by condensation and carbonization, probably during a hydrothermal alteration in small Solar System bodies (Cody et al. 2011). Furthermore, Kebukawa et al. (2013; 2017) illustrated that the presence of ammonia significantly enhances the yield of solid organic matter from aldehydes at 150 °C and simultaneously produced amino acids. Therefore, amino acids,

the building blocks of proteins, may have been synthesized from aldehydes and ammonia. Various organic compounds could be also produced from formaldehyde and ammonia generated from hexamethylenetetramine degradation in environments simulating hydrothermal conditions of asteroids at 150 °C and alkaline pH for 31 days (Vinogradoff et al. 2018). Therefore, organic compounds, including amino acids, may have been synthesized from aldehydes and ammonia in aqueous environments in small bodies.

Minerals could affect hydrothermal reactions of organic matter. The presence of phyllosilicates could catalyze the organic compounds to be more abundant and more complex, and the nature of the phyllosilicate impacted the abundance and the molecular composition of the final organic compounds, including amino acids (Vinogradoff et al. 2020a; 2020b). The different chemical composition of the phyllosilicates suggests different oxidation states, as well as the release of different cations in solution by dissolution, possibly leading to the formation of various complexes rich in organic matter, and, in turn, different catalyzing/inhibiting reactions (Vinogradoff et al. 2020a). Thus, the production of the amino acids could be affected by the unique characteristics of phyllosilicates, such as structure, chemical composition, and crystal size. Yamashita and Naraoka (2014) demonstrated that the presence of olivine powder promoted the formation of alkylpyridines from aldehydes and ammonia (the Chichibabin synthesis). Overall, the presence and the nature of the silicates could affect the abundance and molecular composition of the final organic compounds in primitive bodies during hydrothermal alteration.

1.8. Stability of Amino Acids in Hydrothermal Environments

Amino acids could be synthesized during an aqueous alteration in meteorite parent bodies; however, continual heating may have led to the decomposition of amino acids. It is known that less altered chondrites contain larger amounts of amino acids compared to highly altered or thermally metamorphosed chondrites, in which amino acids decay into monocarboxylic acids through the loss of their amine functional group (Elsila et al. 2016). The stability of amino acids has been studied in simulated submarine hydrothermal vents (e.g., Bada et al. 1995; Kohara et al. 1997; Andersson and Holm 2000; Cox and Seward 2007; Kurihara et al. 2012a; Chandru et al. 2013). Kurihara et al. (2012a) synthesized complex compound amino acid precursors from a primitive terrestrial atmosphere simulated by proton irradiation to examine their stabilities. Their research showed that the compound amino acid precursors conserve more amino acids than free amino acids after heating in hydrothermal submarine environments. However, stability and alteration processes of extraterrestrial amino acids and/or their

precursors under hydrothermal conditions occurring in meteorite parent bodies have not yet been investigated well. A large amount of amino acids in primitive carbonaceous chondrites exists in the form of amino acid precursors (e.g., Glavin et al. 2010).

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Chapter 2

Alteration and Stability of Complex Macromolecular Amino Acid Precursors in Hydrothermal Environments

Published

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Abstract

The early Solar System comprised a broad area of abiotically created organic compounds, including interstellar organics which were integrated into planetesimals and parent bodies of meteorites, and eventually delivered to the early Earth. In this study, I simulated interstellar complex organic compounds synthesized by proton irradiation of a gas mixture of CO, NH₃, and H₂O, which are known to release amino acids after acid hydrolysis based on Kobayashi et al. (1999) who reported that at the first stage of chemical evolution, the main compounds formed abiotically are complex organic compounds with high molecular weights. I examined their possible hydrothermal alteration and stabilities as amino acid precursors under high temperature and pressure conditions simulating parent bodies of meteorites by using an autoclave. I reported that all samples treated at 200–300 °C predominantly released glycine and alanine, followed by α -aminobutyric acid, and serine. After heating, amino acid concentrations decreased in general; however, the recovery ratios of γ -aminobutyric acid increased with temperature. The interstellar complex organic analog could maintain as amino acid precursors after being treated at high temperature (200–300 °C) and pressure (8–14 MPa). However, the molecular structures were altered during heating to form organic compounds that are more stable and can survive in elevated hydrothermal conditions.

2.1. Introduction**2.1.1. Sources of organic matter in early Earth**

The study of the origin of life, besides the evolution of the Earth's environments, helps us realize why the Earth became suitable to live in and why terrestrial life has continued for billions of years. Endogenous synthesis and exogenous delivery of organic compounds are suggested to be two sources of the chemical evolution of the early solar system, and organic compounds delivered by either source are considered necessary for the origin of life.

Endogenous supply of organic compounds

Many experiments have been conducted, including the historic spark discharge experiment, the Miller–Urey experiment, to reconstruct the origin of life on Earth. Miller proved that under highly reduced conditions, various organic compounds, including amino acids, could be obtained by spark discharges (Miller 1953; Miller and Urey 1959). Other investigators showed that bioorganic compounds could be synthesized from strongly reducing starting mixtures using heat (Harada and Fox 1964), UV radiation (Sagan and Khare 1971; Bar-Nun and

Hartman 1978), shock waves (Bar-Nun et al. 1970), and laser-induced plasma energy (Scattergood 1989). Moreover, volcanic outgassing and hydrothermal vents provide alternative sites for organic synthesis (Yanagawa and Kobayashi 1989). On the other hand, it was suggested that they were minor contributors for organic synthesis, for example, submarine hydrothermal vents do not seem to presently synthesize organic compounds more complex than simple hydrocarbons such as CH₄ and ethane (McCollom et al. 2010). It was also claimed that they decompose them over short time spans ranging from seconds to a few hours (Cleaves 2012). Prebiotic formation of bioorganics in hydrothermal vents is still controversial.

However, the predominant view in the last several years has been that the primitive Earth atmosphere was only “weakly reducing”, composed of a mixture of carbon dioxide, nitrogen, and water, coupled with small quantities of carbon monoxide and hydrogen (Walker 1977; Kasting and Brown 1998). Given this, the Miller–Urey-type synthesis in the primitive Earth atmosphere would have been only a minor contributor to the formation of prebiotic organic compounds (Stribling and Miller 1987). Kobayashi and coworkers showed that amino acid precursors could be synthesized from weakly reducing gas mixtures (e.g., carbon dioxide, carbon monoxide, nitrogen, and water) by irradiation with high-energy protons (Kobayashi et al. 1998; Kobayashi et al. 1999). Thus, endogenous production of organic compounds in a weakly reducing atmosphere could be possible by the action of cosmic rays, but their production rate might have been limited.

Extraterrestrial delivery of organic compounds

Extraterrestrial infall through comets, meteorites, and interplanetary dust particles supplied considerable amounts of organic carbon to the surface of the Earth (Oró 1961; Anders 1989; Chyba and Sagan 1992; Pizzarello and Shock 2010). Carbonaceous chondrites, a primitive subgroup of stony meteorites, are mainly composed of silicates but contain abundant carbon (up to 4%) which is mostly present as organic materials. The Murchison meteorite is the most well-studied carbonaceous chondrite for organic matter which is classified as insoluble macromolecular materials (~70%) and simpler soluble compounds (~30%) such as amino acids and carboxylic acids (e.g., Pizzarello et al. 2006). Murchison extracts contain over one hundred amino acids including α -, β -, γ -, δ -, and ϵ -amino acids, and eight of these amino acids are also found in proteins, namely glycine (Gly), alanine (Ala), proline, valine, leucine, isoleucine, aspartic acid (Asp), and glutamic acid (Glu), in addition to numerous other compounds encountered in terrestrial metabolisms (Pizzarello and Shock 2010, and references there in).

Many experiments have been conducted with energy sources in the extraterrestrial environments to synthesize hydrocarbons, amino acids, and nucleic acid bases from simple molecules. A possible energy source for the formation of complex organic molecules could be an ultraviolet irradiation of dust grains in the interstellar medium. Muñoz Caro et al. (2002) performed simulation of interstellar ices mixtures of water, carbon dioxide, methanol, and ammonia, placed under a pressure of 10^{-7} mbar at 12 K and irradiated with electromagnetic radiation, as well as their analysis by gas chromatography/mass spectrometry. They identified 16 amino acids, in the simulated ice mantle of interstellar dust particles, including 6 protein amino acids. Moreover, vacuum UV photoirradiation of simpler ices containing water, methanol, and ammonia led to the formation of hydantoin, 2,4-imidazolidinedione (De Marcellus et al. 2011). The dominant amino acids were formed when 5-substituted hydantoins were UV-irradiated and hydrolyzed were Gly and Ala (Sarker et al. 2013). Kobayashi and coworkers simulated interstellar ice by using a starting material composed of a mixture of methanol, ammonia, and water (molar ratio = 1:1:2.8) and irradiated by heavy ions, and they hydrolyzed amino acids by using an ion-exchange high-performance liquid chromatography (IE-HPLC). A wide range of amino acids were detected after acid hydrolysis, including protein amino acids such as Ala, Gly, and Asp as well as non-protein amino acids such as β -alanine (β -Ala) and α - and γ -aminobutyric acid (Kobayashi et al. 2012). Another possible scenario of the formation of organic matter is via aqueous alteration or a hydrothermal reaction in the meteorite parent bodies. A possible reaction for the formation of α -amino acids is generally considered to be a Strecker-like reaction from precursor aldehydes, ketones, ammonia, and HCN in the presence of water. On the other hand, macromolecular organic solids can be produced from hydrothermal reaction of formaldehyde and yielded organic solids (Cody et al. 2011), and α -, β -, and γ -amino acid precursors were produced simultaneously with organic solids when ammonia was added in the hydrothermal reaction of formaldehyde (Kebukawa et al. 2017).

2.1.2. Stability of amino acids in hydrothermal environments

Although amino acids could be synthesized during an aqueous alteration in meteorite parent bodies, continuous heating may have resulted in the decomposition of amino acids. It is known that less altered chondrites contain larger amounts of amino acids compared to highly altered or thermally metamorphosed chondrites, in which amino acids decay into monocarboxylic acids through the loss of their amine functional group (Elsila et al. 2016). So far, the stability of amino acids in hydrothermal conditions has been studied in simulated submarine

hydrothermal vents (e.g., Andersson and Holm 2000; Bada et al. 1995; Chandru et al. 2013; Cox and Seward 2007; Kohara et al. 1997; Kurihara et al. 2012a). Kurihara et al. (2012a) tested the stability of complex combined amino acid precursors, which were synthesized from a simulated primitive Earth atmosphere with proton irradiation, and showed that the complex combined amino acid precursors preserved more amino acids than free amino acids after heating in a simulated submarine hydrothermal environments. However, stability and alteration processes of extraterrestrial amino acids and/or their precursors under hydrothermal conditions occurring in meteorite parent bodies have not yet been investigated well. A large amount of amino acids in primitive carbonaceous chondrites exists in the form of amino acid precursors (Glavin et al. 2010). In this study, I tested the stability and decomposition of the interstellar complex amino acid precursor analog during hydrothermal alteration simulating meteorite parent bodies under elevated temperatures.

2.1.3. Hydrothermal systems

In the late '70s, the discovery of hydrothermal systems introduced a new theory to the origin of life, as they gave us opportunities to study the discovered meteorites in laboratories. In both closed and flow systems, numerous forms of simulations have been carried out since then. hydrothermal systems may be a location where life began on Earth (Corliss et al. 1981), due to its high energy, reduced environments, and the availability of various starting chemicals (i.e. CH₄, C₂H₆, H₂S, CO, and H₂).

Autoclaves, the closed systems, were used as typical instruments to conduct experiments involving hydrothermal systems (Kobayashi et al. 1995; Kohara 1997; Kurihara et al. 2012a). Yanagawa and Kojima (1985) showed that when glycine (0.3 M), alanine (0.1 M), aspartic acid (0.1 M) and valine (0.3 M) were heated in an autoclave at 250°C or 300°C, microsphere-like structures were formed. Though not well-defined, these kinds of structures could still play a role in chemical evolution. In such hydrothermal system, many experiments seem to support the formation of biomolecules, especially amino acids. Hennessey et al (1992) reported that a variety of amino acids could be formed from KCN, NH₄CL and HCHO along with pyrite, pyrrhotite and magnetite at 150°C in an autoclave. Yanagawa and Kobayashi (1992) performed simulation experiments by heating an aqueous solution containing metal mixture and NH₄⁺ in an autoclave at 325°C and pH 3.6 under pressurized gas mixture of CH₄ and N₂, as a result, amino acids such as glycine, alanine, aspartic acid, and serine could be detected. Islam et al. (2002) reported the formation of glycine and alanine after heating a solution mixture of KCN, HCHO and NH₄HCO₃ at 100°C and 10 MPa for 16 hours in the autoclave under pressurized

gas mixture of nitrogen and hydrogen (N₂ 99% and H₂ 1%). The autoclave is preferable to examine the stability of amino acids at high temperatures and pressure for long duration due to its robustness, in addition, it has many parameters which could be used to investigate their effects on the formation and alteration of the amino acids.

In my experiments, I investigated the stability of the amino acid precursors as complex amino acids in environments simulating parent bodies of meteorites and I checked the effects of the elevated temperatures in destroying the produced amino acids, then I investigated the temperatures at which amino acid would have likely been decomposed in parent planetesimals. My data provide insights into the chemistry behind the destruction of these amino acids during the alteration process that happened in parent body-like environments.

2.2. Methods

2.2.1. Materials and Samples

Complex organic compounds were synthesized from a gas mixture of CO, NH₃, and H₂O, which are possible constituents of the interstellar medium, by irradiation with 2.5 MeV protons from a Tandem accelerator (Tokyo Institute of Technology) following the method of Takano et al. (2004). The total energy applied to the gas mixture was 3.16 kJ. The solution of synthesized complex organic compounds is slightly yellow and contains several thousand Daltons of amino acid precursors, which yielded various amino acids (protein and nonprotein amino acids) after acid hydrolysis (Takano et al. 2004). Hereafter, I simply called the amino acid precursor-containing solution synthesized from a gas mixture of CO, NH₃, and H₂O; the “AAP”. I used the AAP as an analog of the primordial organic matter in primitive meteorite parent bodies to evaluate the stability of amino acids and their precursors.

2.2.2. Autoclave Experiments

To simulate hydrothermal conditions in meteorite parent bodies, an autoclave (AC) (Figs. 1, 2), which is a closed hydrothermal system, was used in my hydrothermal experiments (Yanagawa and Kobayashi 1992; Kobayashi et al. 1995; Islam et al. 2002). It uses an electrothermal heater, in which the heating starts from room temperature (RT) to the desired temperature of 200–300 °C. The inside temperature was monitored using thermocouples, and the pressure was maintained constant during the heating experiments.

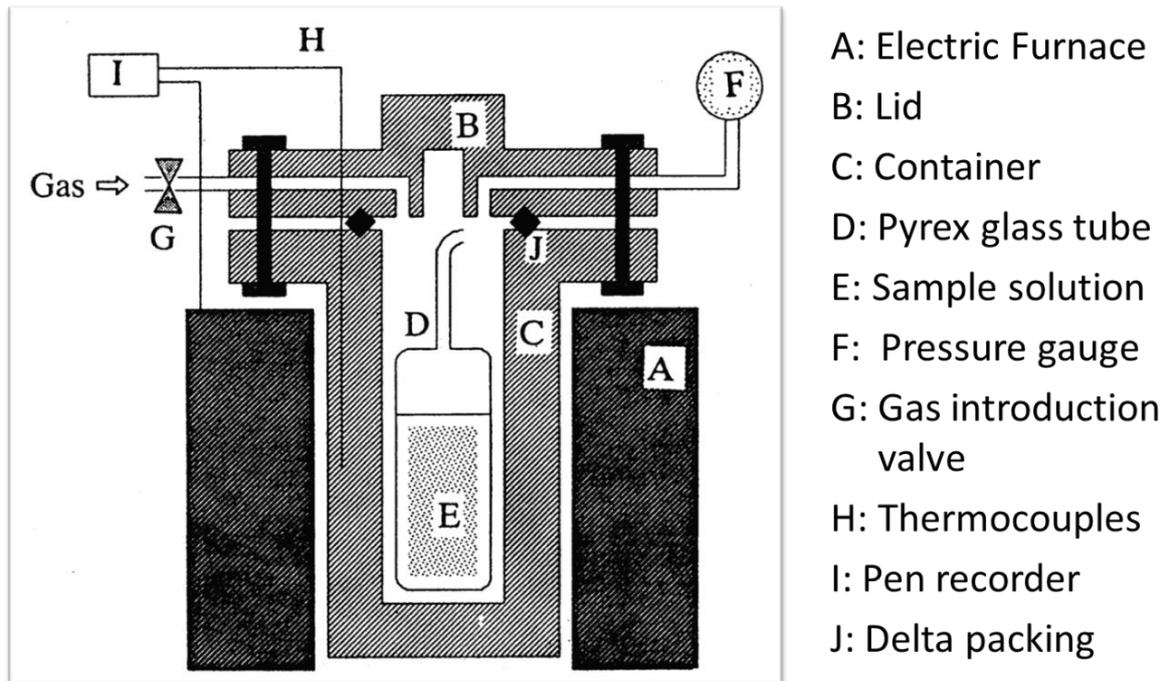


Fig. 2-1 Schematic diagram of Autoclave (AC) (Kobayashi et al. 1997).



Fig. 2-2 Photo for the used autoclave, Yokohama National University.

The experimental procedures are summarized in Fig. 2-3a. The AAP was placed in a reaction tube, then settled the tube in the AC, and the AC lid was closed firmly. To compare the effects of silicate and metal ions, both Pyrex glass tubes and stainless-steel tubes were used as the reaction tubes in this study. The AC was purged with N₂ or a gas mixture of N₂: H₂ (99:1 vol%) to evaluate the effects of redox condition—the pressure valve was opened and closed three times to eliminate all the gas inside the AC and was then pressurized to 8 MPa at RT. The N₂+H₂ gas mixture provides a more reduced condition than pure N₂ gas. The pressure was increased to 14 MPa with continuous heating at high temperatures of 200, 250, and 300 °C. It took up to 30 minutes to reach the target temperatures. After the set temperature was reached, the heating of the AC was continued for 2 h, after which the AC was left to cool down for approximately 30 minutes. A control AAP was also prepared by placing the solution in the AC under the same experimental conditions but without heating or pressurizing. In this research, I could control temperature, pressure, heating period, and metal ions in some cases only but in future work, within a possible range of environments, other parameters would be changed for such range.

2.2.3. HPLC analysis

The procedures for amino acid analysis are summarized in Fig. 2-3b. For amino acid analysis, after each autoclave run, an aliquot (0.5 mL) of the filtrated AAP was mixed with 0.5 mL of 12 M HCl and heated at 110 °C for 24 h for acid hydrolysis. The acid hydrolysis method that was applied here is selected due to: (i) Proteins and meteoritic amino acids (precursors) have been acid hydrolyzed to evaluate amino acid composition by acid hydrolysis in 6 M HCl at 100-110 °C for 24 hours (Cronin and Pizzarello 1997; Simkus et al. 2019), and (ii) The AAP has been evaluated after the same acid hydrolysis conditions and obtained fair results to evaluate the synthetic/alteration reaction conditions. Under the previous classical hydrolysis conditions, the following amino acids; aspartic acid, glutamic acid, proline, glycine, alanine, leucine, phenylalanine, histidine, arginine, and hydroxyproline are stable and they can be determined quantitatively (Davidson 2003; Rutherford and Gilani 2009). The remaining amino acids have some exceptions, for example, asparagine and glutamine are hydrolyzed quantitatively to aspartic acid and glutamic acid, as well as, there are some losses in the remaining amino acids. It should be noted that all amino acid recovery ratios in my experiments were evaluated after acid hydrolysis in the same conditions, thus the influence of acid hydrolysis would be minimum for comparison of recovery ratios after the AC experiments.

After acid hydrolysis, the AAP was dried by centrifugation at 60°C. The hydrolyzed fraction undergoes to desalting with cation-exchange resin (AG-50W-X8 resin) after dissolving in 2 mL of 0.1 M HCl. The resin (2–3 mL) was prewashed using the following liquids sequentially: 1 M HCl (20 mL), water (20 mL), 1 M NaOH (20 mL), water (20 mL), 1 M HCl (20 mL), and water (20 mL). An aliquot of 0.1 M HCl sample solution (1 mL) was introduced to the column after filtration with polytetrafluoroethylene (PTFE) membrane filters (DISMIC-13HP 0.45 µm) and rinsed with 0.1 M HCl (1 mL) and water (20 mL). Then, the solution containing amino acids was eluted with 10% NH₃ aqueous solution (15 mL) and water (20 mL). The NH₃ elute was dried by centrifugation at 60°C, and the dried sample was dissolved in 0.5 mL of Milli-Q water. All the glassware used was baked at 500 °C for at least 3 h before use in all experiments.

Before injection to the HPLC system, all the AAP samples were filtrated through cellulose acetate membrane filters (DISMIC-3CP 0.45µm). A 100-µL aliquot of each AAP solution was subjected to amino acid analysis by using an IE-HPLC system. The HPLC system was equipped with a system controller (Shimadzu SCL-10A), three HPLC pumps (two Shimadzu LC-20AD for carriers, and one LC-10ATvp for derivatization reagent) and a polystyrene-type ion-exchange column (Shimadzu Shim-pack ISC-07/S1504Na; 4.0 mm of inner diameter × 150 mm; particle diameter of 7 µm). The column temperature was conserved at 55 °C by a column heater (Sugai U-620 type 50). A fluorescence detector (Shimadzu RF-535) with an excitation wavelength of 355 nm and an emission wavelength of 435 nm was used. A post-column derivatization was used with a solution of o-phthalaldehyde (OPA, 0.104 g/L) and N-acetyl-L-cysteine (NAC, 0.65 g/L), Na₂CO₃ (40.7 g/L), H₃BO₃ (13.5 g/L), K₂SO₄ (18.8 g/L), and polyoxyethylene lauryl ether (0.2 g/L). Gradient elution was performed using a Shimadzu amino acid mobile phase kit (Na type): (A) 0.07 M sodium citrate, hydrochloric peroxide, with 7% ethanol (pH 3.2); (B) 0.2 M sodium citrate, boric acid, and sodium hydroxide (pH 10); and (C) 0.2 M sodium hydroxide. The flow rate of the carrier was 0.3 mL/min. For peak identification, commercial amino acid standard solutions (Wako Amino Acids Mixture Standard Solution, Type B, and Type AN-2) were used.

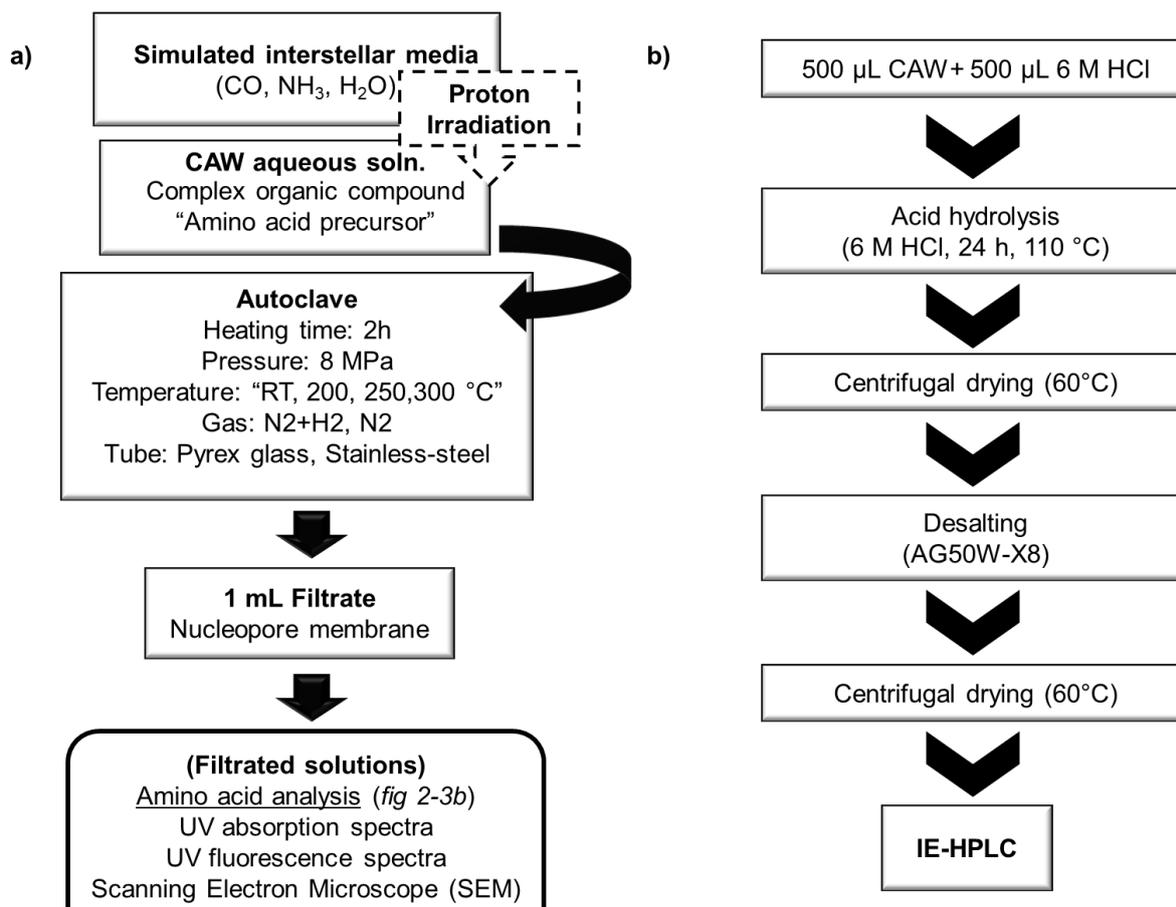


Fig. 2-3 (a) Summary of experimental flows, and (b) Summary of amino acid analysis procedure by IE-HPLC.

2.2.4. UV absorption and UV fluorescence spectra

Ultraviolet-visible (UV-Vis) spectroscopy was performed to investigate the electronic energy levels changes within the molecule arising due to the transfer of electrons from π - or non-bonding orbitals. It usually provides information about π -electron systems, conjugated unsaturation, aromatic compounds, and conjugated non-bonding electron systems. This absorption spectroscopy uses electromagnetic radiations of wavelength 190–800 nm, which is divided into the UV (190–400 nm) and Vis (400–800 nm) regions.

An aliquot (0.7 mL) of each filtrated AAP was analyzed using a UV-VIS spectrometer (JASCO V-660). The absorption spectra were obtained with a wavelength range of 187–900 nm using a deuterium lamp (187–350 nm) and a halogen lamp (350–900 nm), with a wavelength accuracy of ± 1.5 nm and a spectral bandwidth of 2 nm. The optical system was a double-beam with a double monochromator to provide higher resolution with extremely low

stray light (0.00008%) to confirm more accurate measurement over a wide photometric range. The UV absorption properties of the AAP were shown for a wavelength range of 200–400 nm.

The fluorescence intensities of 1 mL of the filtrated AAP were analyzed using a spectrofluorometer (JASCO FP-6300), equipped with a 150-watt Xe light source, a silicon photodiode detector for excitation monochromator, and a photomultiplier for the emission monochromator. The wavelength range was 220–700 nm with a standard photomultiplier tube, a wavelength accuracy of ± 2 nm, and a spectral bandwidth of 20 nm for both excitation and emission monochromators. The UV fluorescence intensities of the AAP were determined at an excitation range of 220–600 nm and an emission range of 230–700 nm.

2.2.5. Scanning Electron Microscope (SEM) analysis

The heated AAP sample solutions were filtered onto 0.2 μm pore size isopore polycarbonate membrane filters (ADVANTEC) and the filters were observed with a scanning electron microscope (KEYENCE KE-8800).

2.3. Results

2.3.1. Amino acid concentrations

The concentrations of amino acids after acid hydrolysis of the heated AAP were quantified using HPLC. Figures 2-4 and 2-5 show chromatograms of the control AAP treated at RT, and the heated AAP at 200, 250, and 300 °C under N_2 and N_2+H_2 in Pyrex glass tubes. A wide range of amino acids were detected after acid hydrolysis (Table 3-1). The control AAP exhibited the following amino acids: Gly and Ala with minor amount of Asp, Glu, serine (Ser) as well as non-protein amino acids; β -Ala and γ -aminobutyric acid. For all the heated AAP at different temperatures (200, 250, and 300 °C), Gly and Ala were predominant, followed by α -aminobutyric acid, Ser and Glu. After heating, amino acid concentrations decreased in general, but some amino acids increased with temperature. The recovery ratios (%) of amino acids were obtained by the amino acid abundances from the heated AAP divided by those from the control AAP (treated at RT under the same experimental conditions) (Fig. 2-6). For the heated AAP under a gas mixture of N_2+H_2 , Gly and Ala showed relatively high recovery ratios (40%) at 200 °C; this recovery ratio continued to decrease with increasing temperature and became only trace amounts at 300°C. Asp and γ -aminobutyric acid showed different responses with heating compared to Gly and Ala. Asp showed a recovery ratio of 40% at 200 °C, which continued to increase and became 60% at 300 °C. γ -aminobutyric acid showed a high recovery ratio of 189%

at 200 °C, which increased efficiently to become 422% at 250 °C, but finally, decreased to 144% at 300 °C.

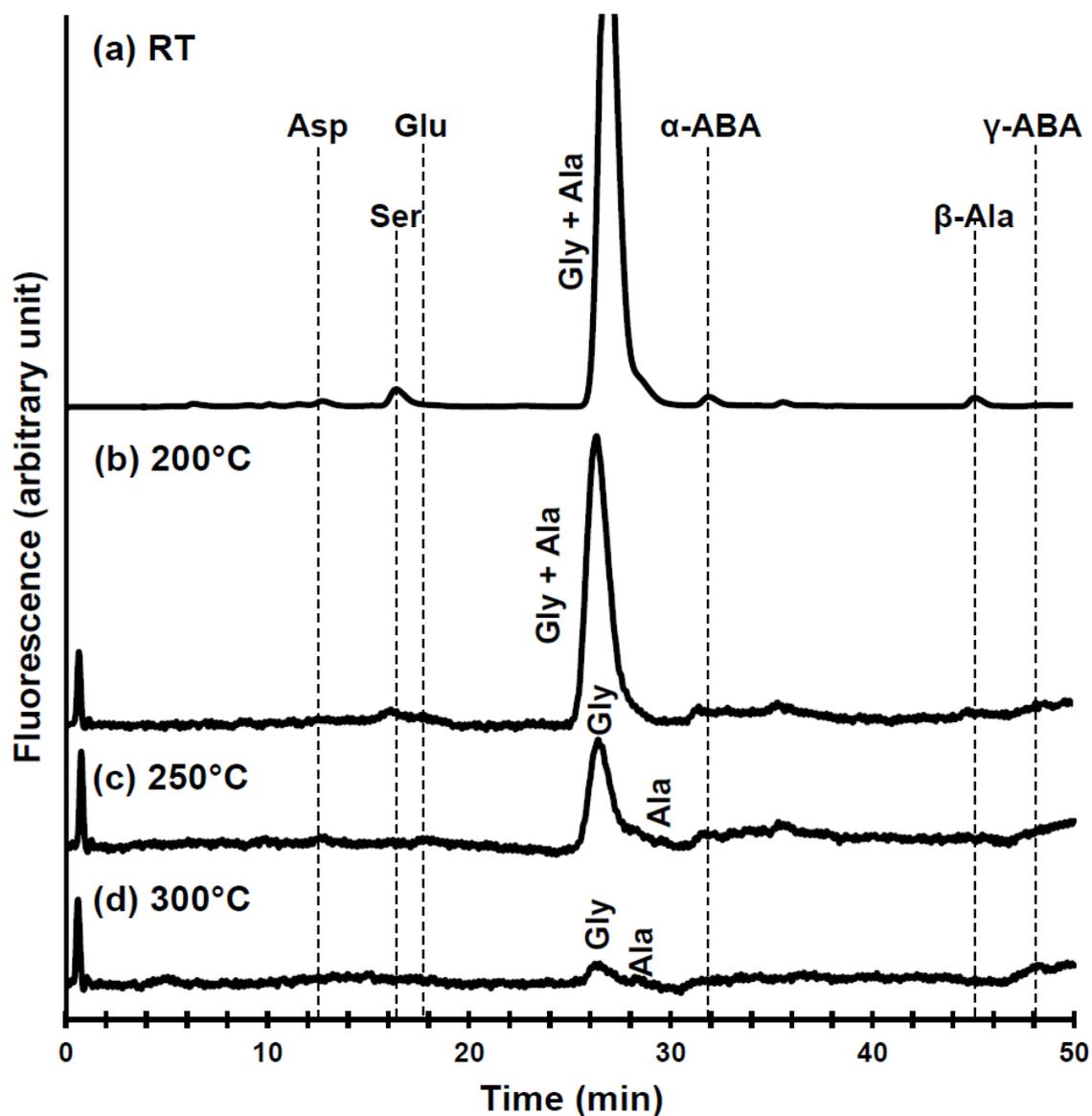


Fig. 2-4 Chromatograms of the acid-hydrolyzed AAP that treated in AC under N₂ gas using Pyrex glass tube at (a) room temperature (control), (b) 200°C, (c) 250°C, and (d) 300°C. (Note: Asp: aspartic acid, Ser: serine, Glu: glutamic acid, Gly: glycine, Ala: alanine, α-ABA: α-aminobutyric acid, β-Ala: β-alanine, γ-ABA: γ-aminobutyric acid)

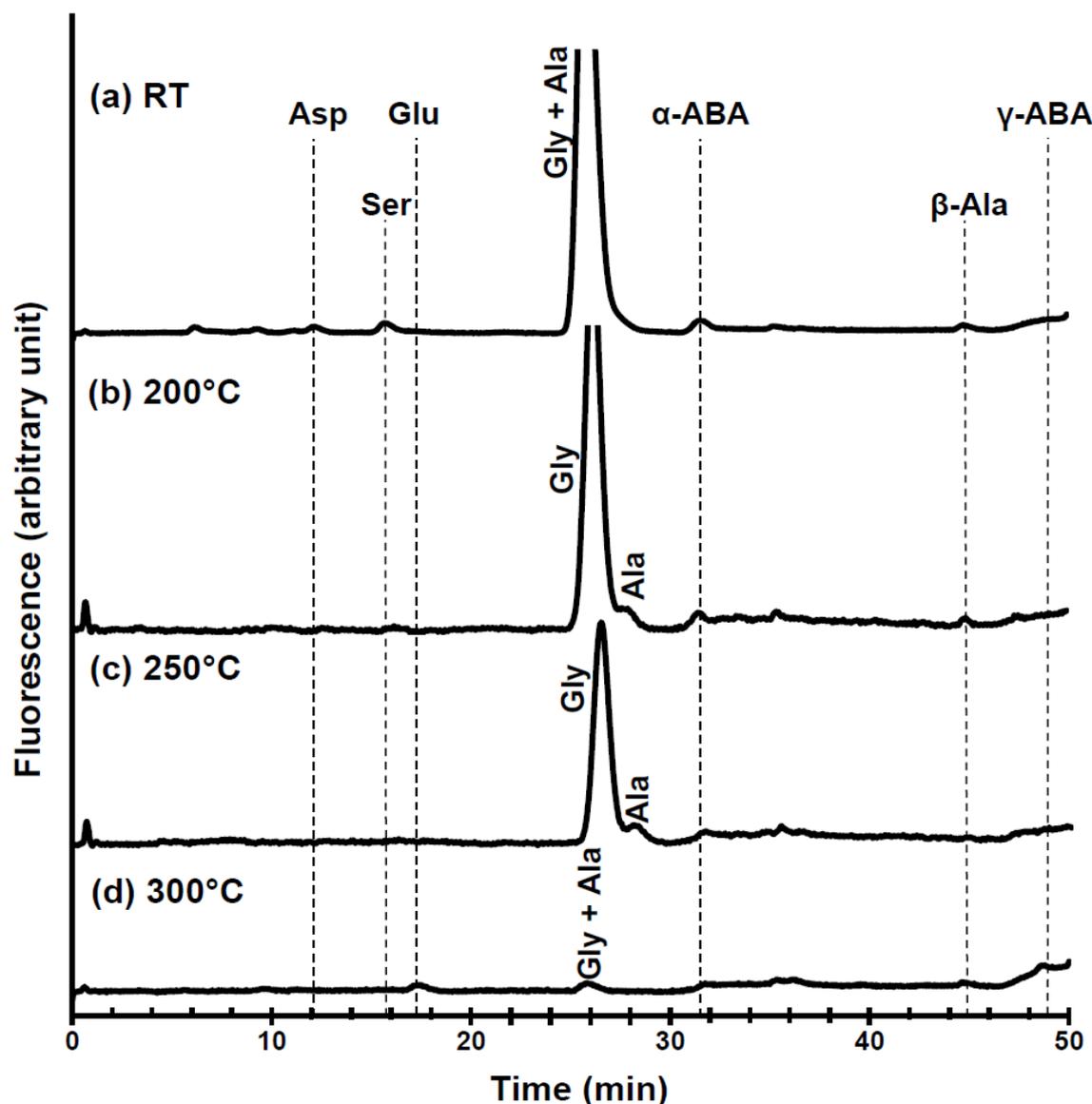


Fig. 2-5 Chromatograms of the acid-hydrolyzed AAP that treated in AC under a gas mixture of N_2 and H_2 (99:1) using Pyrex glass tube at **(a)** room temperature (control), **(b)** 200°C, **(c)** 250°C, and **(d)** 300°C. (Note: Asp: aspartic acid, Ser: serine, Glu: glutamic acid, Gly: glycine, Ala: alanine, α -ABA: α -aminobutyric acid, β -Ala: β -alanine, γ -ABA: γ -aminobutyric acid)

For the heated AAP under N_2 gas, amino acids showed relatively lower recovery ratios than the heated AAP under a gas mixture of N_2+H_2 , although they behave almost in the same manner in response to heating. For instance, the recovery ratios of Gly and Ala at 200 °C were 34% and 23%, respectively, whereas their recovery ratios were greatly decreased and became 0.38% and 0.25%, respectively, at 300 °C. Moreover, the recovery ratio of Asp constantly increased from 33% at 200 °C to 43% at 300 °C. Finally, γ -aminobutyric acid displayed again

the same behavior of an increased recovery ratio from 80% at 200 °C to 87% at 250 °C, then a decreased ratio of 44% at 300 °C.

Gly and Ala decreased constantly with increasing temperature, but some amino acids showed a different behavior, such as Asp and γ -aminobutyric acid, which increased with increasing temperature in some cases. At all temperatures, the recovery ratio of each amino acid increased when hydrogen was added to the pressurizing gas.

Table 2-1. Amino acid concentrations and recovery ratios in the control AAP and the heated AAP after acid hydrolysis.

Concentration (μ M)	N ₂ , Pyrex			N ₂ + H ₂ , Pyrex				
	Control	200 °C	250 °C	300 °C	Control	200 °C	250 °C	300 °C
Glycine	494.3	168.1	39.3	1.9	525.2	210.3	129.0	2.1
Alanine	126.1	28.9	0.4	0.3	154.4	61.8	5.9	2.0
β -alanine	2.7	0.4	0.2	0.1	3.0	1.1	0.6	0.3
Aspartic acid	3.9	1.3	1.2	1.7	4.3	1.7	2.0	2.6
Serine	5.7	1.0	0.2	0.1	7.7	2.3	0.8	0.8
α -aminobutyric acid	7.8	4.0	2.8	0.9	10.9	12.0	8.2	2.2
γ -aminobutyric acid	0.7	0.5	0.6	0.3	2.0	3.8	8.4	2.9
Recovery ratio (%)								
Glycine	34	8.0	0.38	0.38	40	25	0.40	0.40
Alanine	23	0.34	0.25	0.25	40	3.8	1.3	1.3
β -alanine	14	7.5	5.4	5.4	36	19	10	10
Aspartic acid	33	30	43	43	40	46	60	60
Serine	17	3.9	1.9	1.9	30	10	10	10
α -aminobutyric acid	51	36	12	12	110	75	20	20
γ -aminobutyric acid	80	87	44	44	189	422	144	144

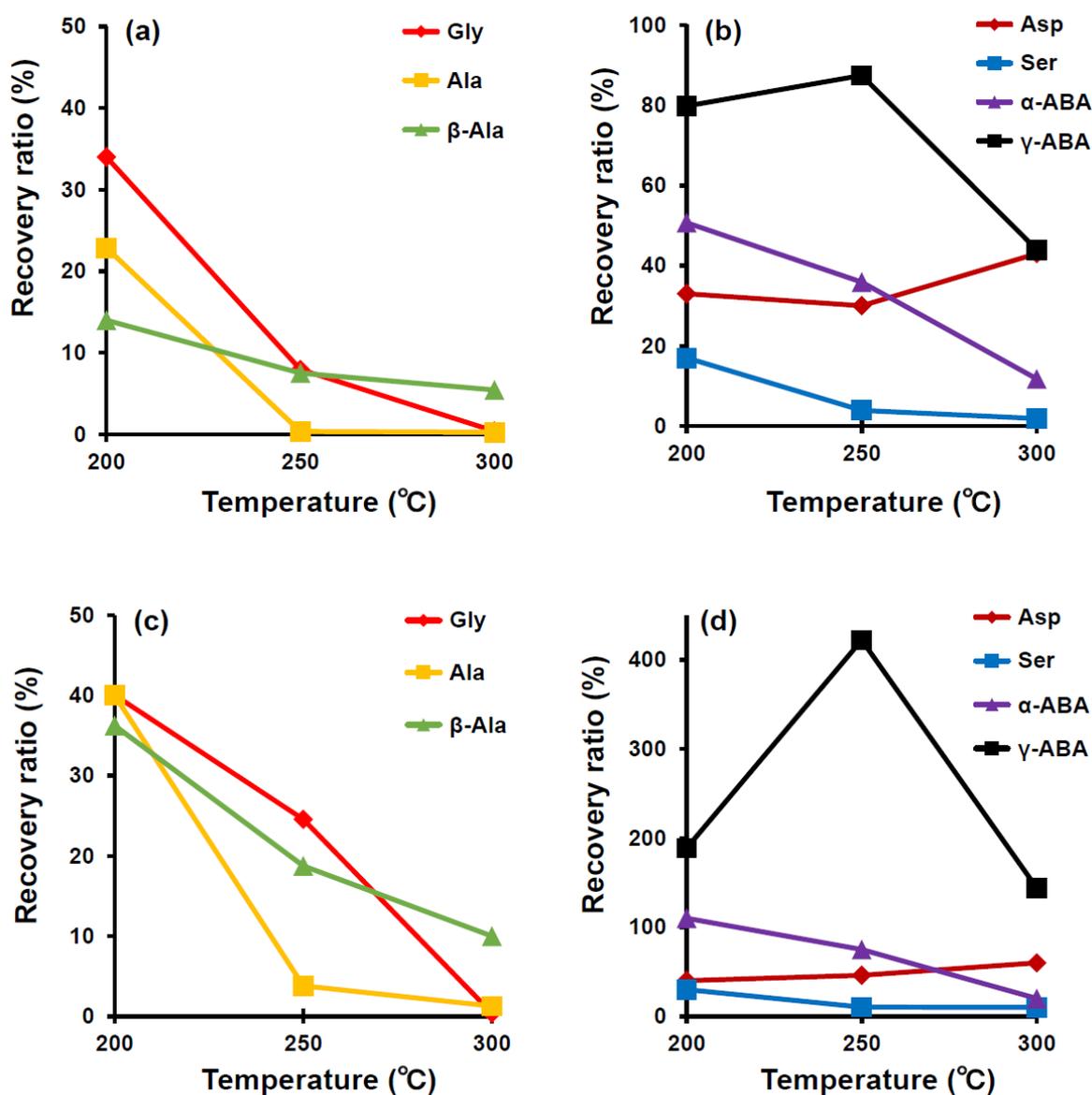


Fig. 2-6 Recovery ratios of amino acids in AAP samples that treated in AC using Pyrex glass tube at 200°C, 250°C, and 300°C under N₂ gas (a,b), and under a gas mixture of N₂ and H₂ (c,d).

2.3.2. UV absorption and UV fluorescence spectra

Figure 2-7 shows the UV absorption spectra of the untreated AAP and the AAP after heating. The UV absorption from 200 to 400 nm was mainly based on the presence of the aromatic carbon structure, while at 226–250 nm were mainly caused by unsaturated π - π^* transitions of the aromatic C=C bond, while wide absorption peaks around 250–400 nm were due to the benzene ring structures with multiple conjugated structures n - π^* and

$\pi-\pi^*$ transitions. Both transitions require the presence of an unsaturated functional group (chromophore), such as $>C=C$, $>C=O$, $-N=O$, $>C=N-$, $-N=N-$, $>C=N-$, to provide the π orbitals.

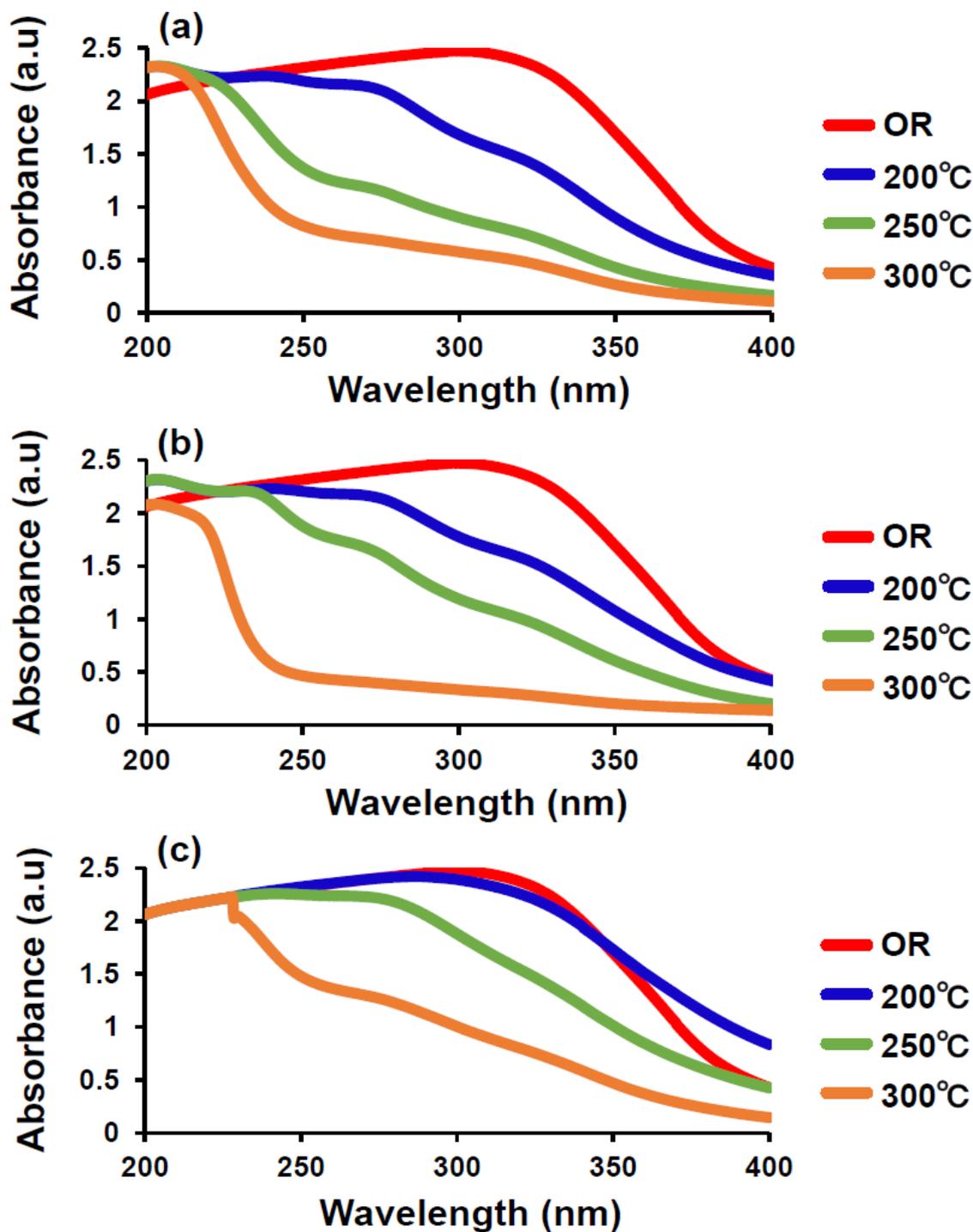


Fig. 2-7 UV absorption spectra of AAP heated in (a) Pyrex glass tubes under N₂ gas, and (b) Pyrex glass tubes under a gas mixture of N₂+H₂, and (c) stainless steel tubes under a gas mixture of N₂+H₂. OR: the untreated original AAP.

Subsequently, a broad peak is observed in the 250–300 nm region composed of multiple overlapping bands from the aromatic structures. An absorption peak at 300–400 nm corresponds to $n-\pi^*$ transitions of the C=O bond. The absorbance at the 220–350 nm region decreased as the temperature increased, and this behavior was almost the same for heating under N_2 and N_2+H_2 in Pyrex glass tubes. In the case of heating in stainless steel tubes, although UV absorption of the AAP decreased with increasing temperature, each absorbance was higher than the case of heating in the glass tube.

In fluorescence analyses, a sample absorbs a photon to move to an excited state and emits a photon to return to the ground state. The intensity of the fluorescence is generally proportional to the concentration of organic molecules. Figure 2-8 shows a three-dimensional UV fluorescence spectrum of the untreated AAP. All spectra are plotted on the same emission/excitation wavelengths and intensity scales to allow comparison. Typically, aromatic molecules exhibit strong and characteristic fluorescence when excited with UV. Figures 2-9–2-11 show three-dimensional UV fluorescence spectra of the heated AAP. Figure 2-12 shows the peak intensities of the UV fluorescence spectra of the AAP, that show a slight increase with increasing the temperature.

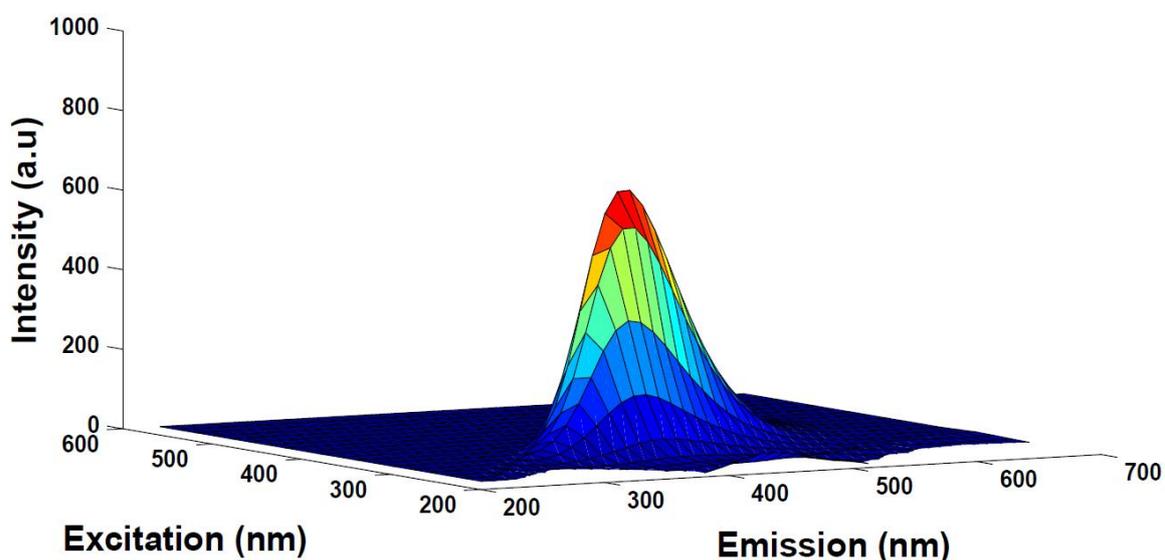


Fig. 2-8 Three-dimensional UV fluorescence spectrum of untreated AAP.

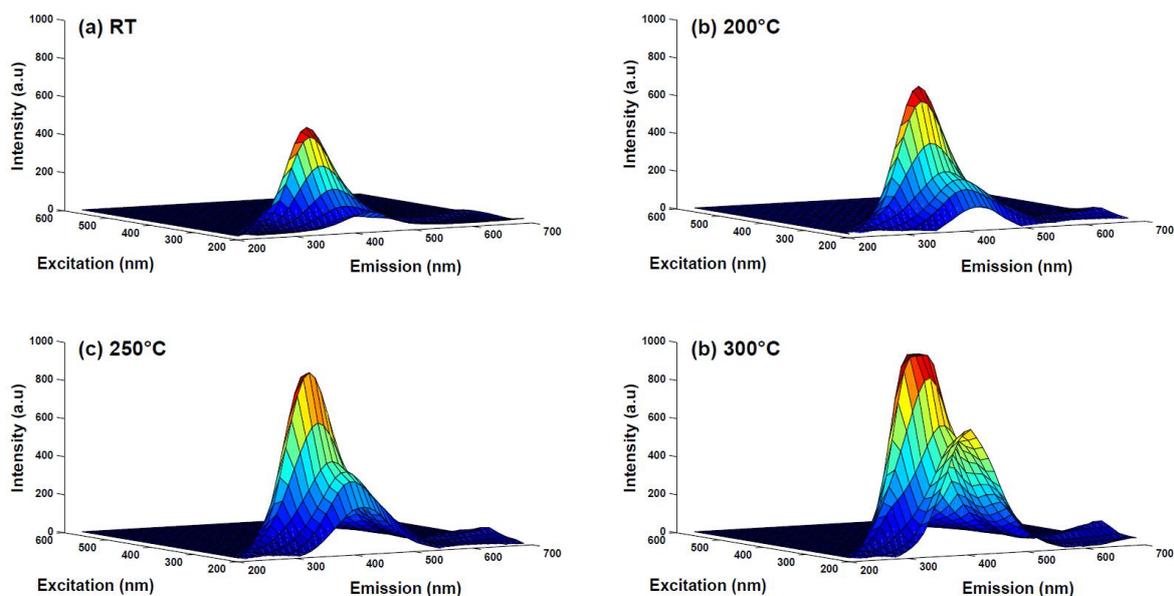


Fig. 2-9 Three-dimensional UV fluorescence spectra of AAP samples that treated in AC using Pyrex glass tube under N₂ gas at (a) room temperature (control), (b) 200°C, (c) 250°C, and (d) 300°C.

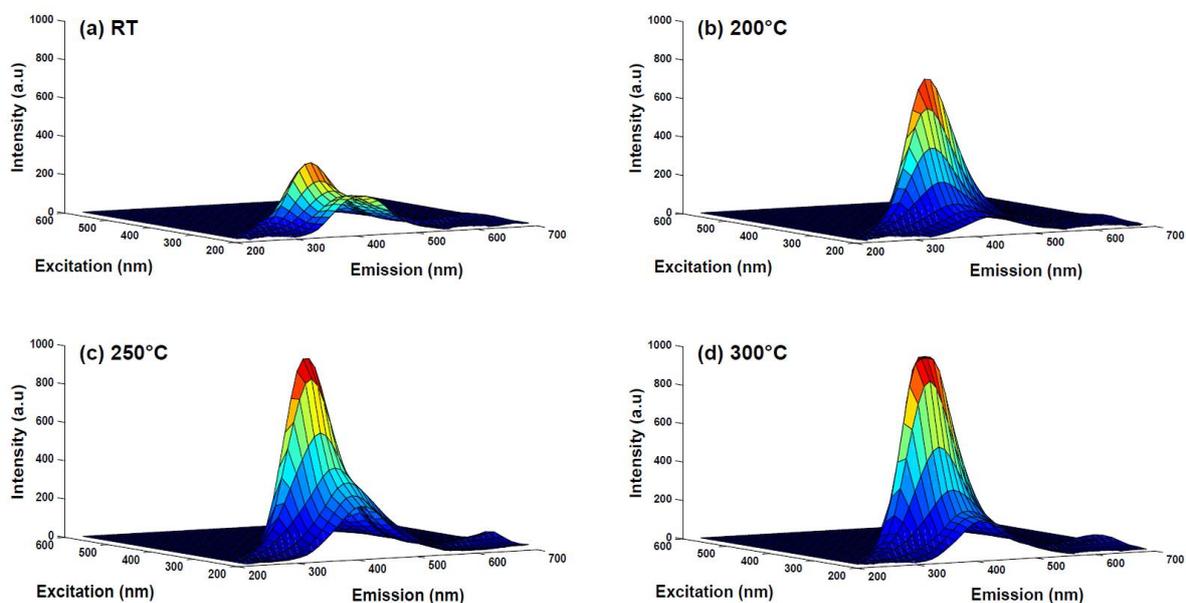


Fig. 2-10 Three-dimensional UV fluorescence spectra of AAP samples that treated in AC using Pyrex glass tube under a gas mixture of N₂+H₂ at (a) room temperature (control), (b) 200°C, (c) 250°C, and (d) 300°C.

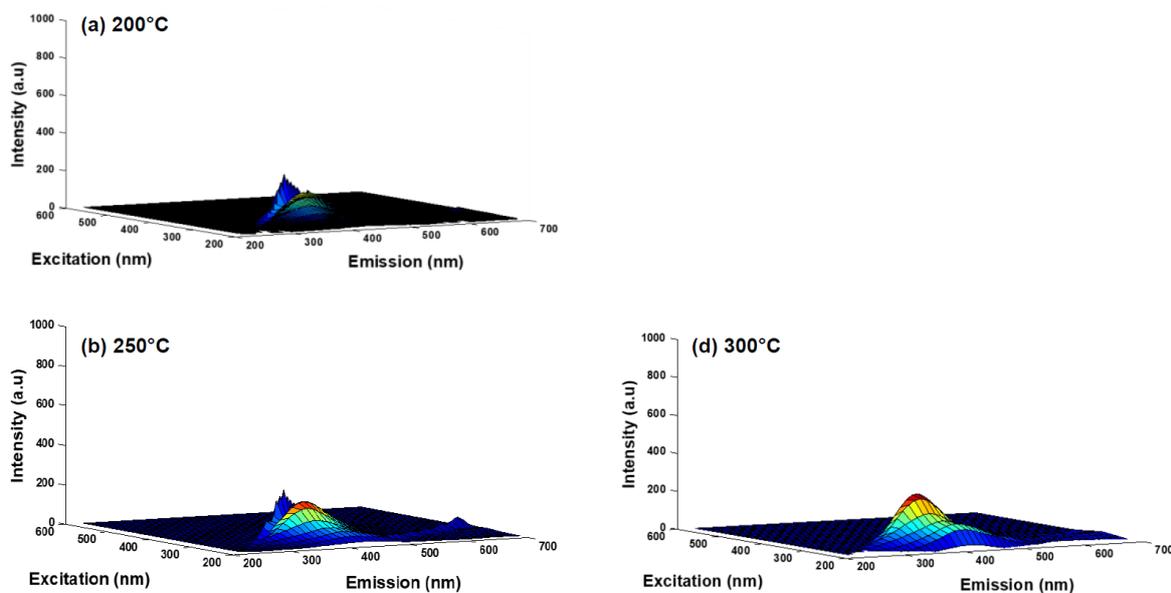


Fig. 2-11 Three-dimensional UV fluorescence spectra of AAP samples that treated in AC using stainless steel tube under a gas mixture of N₂+H₂ at (a) 200°C, (b) 250°C, and (c) 300°C.

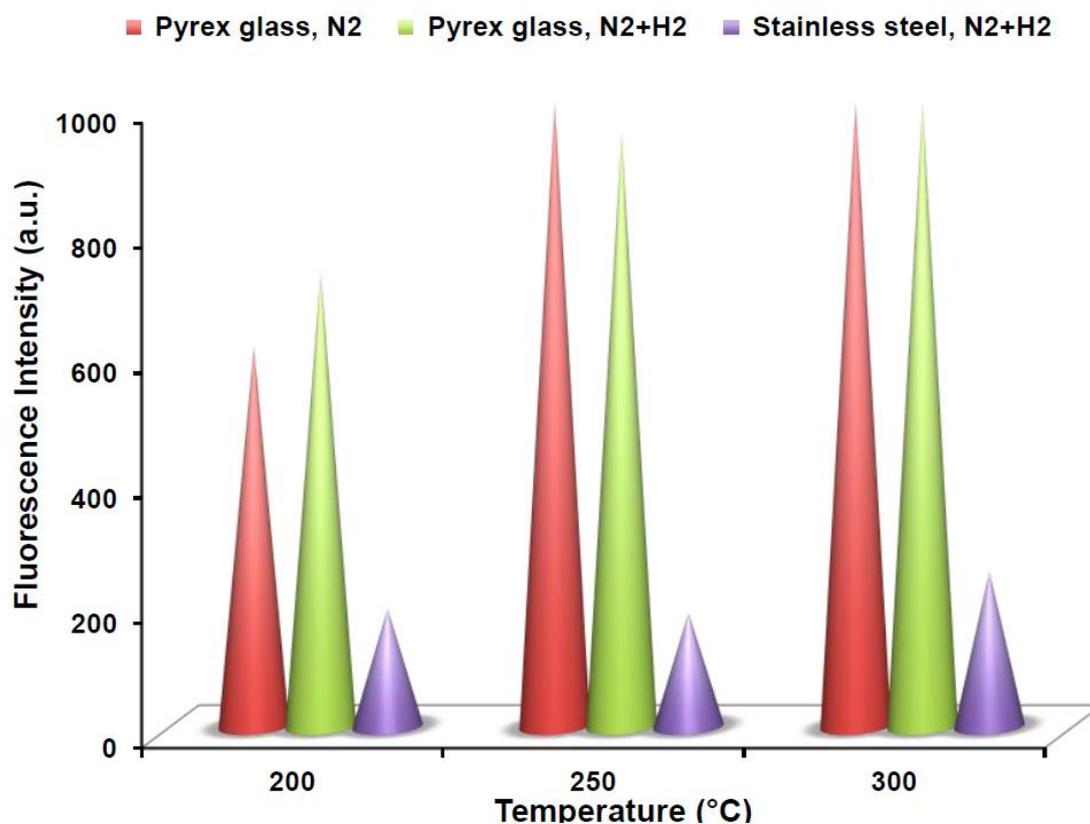


Fig. 2-12 Fluorescence intensity of AAP samples that treated in AC at 200, 250 and 300°C, in Pyrex glass tubes under a N₂ gas, in Pyrex glass tubes under a gas mixture of N₂+H₂, or in stainless steel tube under a gas mixture of N₂+H₂.

2.3.3. Scanning Electron Microscope (SEM) analysis

Figures 2-13 and 2-14 showed AAP original sample and AAP after heating in Pyrex glass tubes and stainless steel tubes under a gas mixture of N_2+H_2 . SEM images of the heated AAP in both types of tubes, Pyrex glass and stainless steel tubes showed formation of the aggregates which increased with increasing the heating temperature, whereas no aggregates were observed in the original sample.

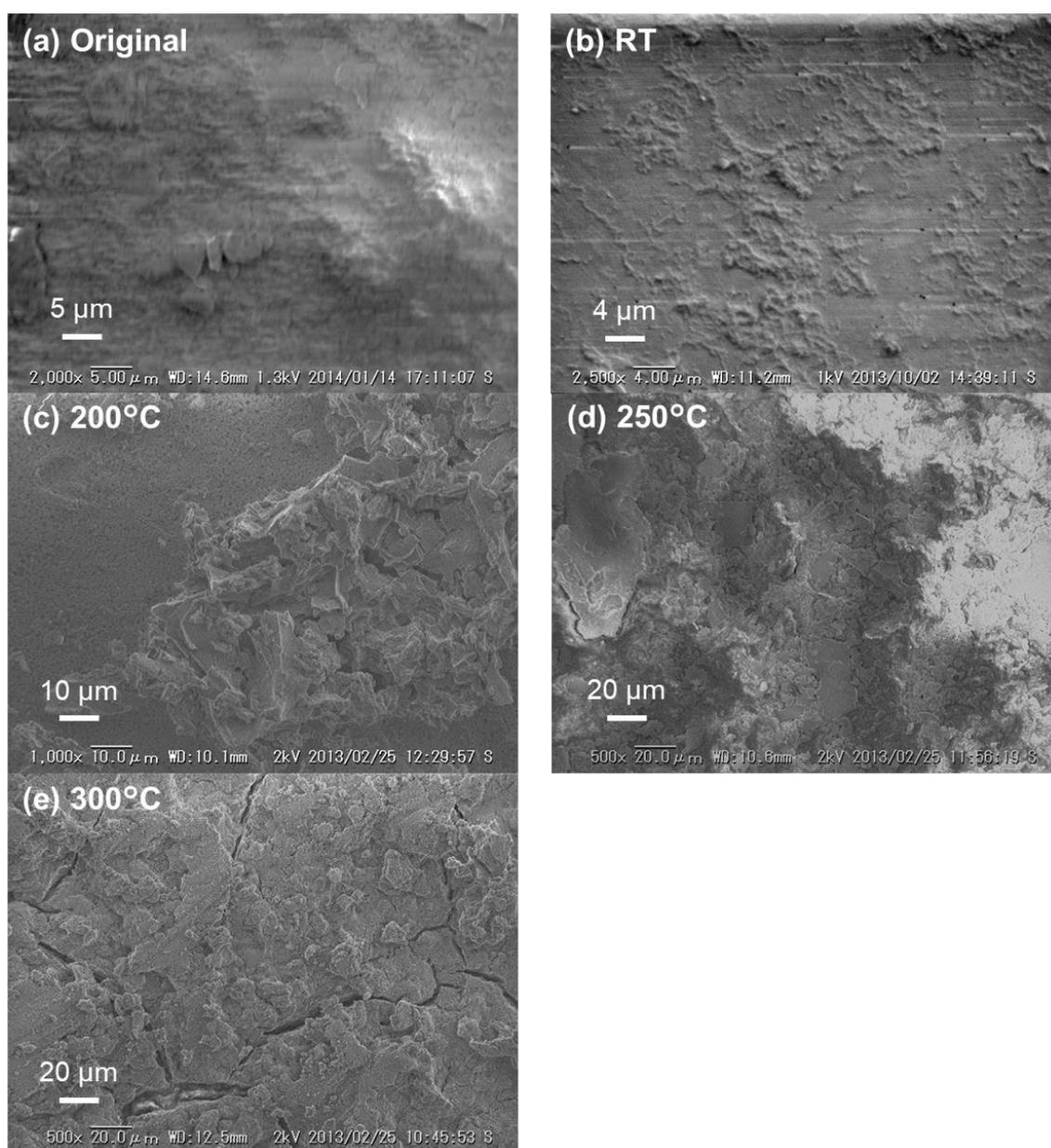


Fig. 2-13 SEM images of AAP samples. (a) original samples without any treatment, (b) control sample treated in AC with Pyrex glass tube under a gas mixture of N_2+H_2 at room temperature, (c-e) heated at (c) 200 $^{\circ}C$, (d) 250 $^{\circ}C$, and (e) 300 $^{\circ}C$

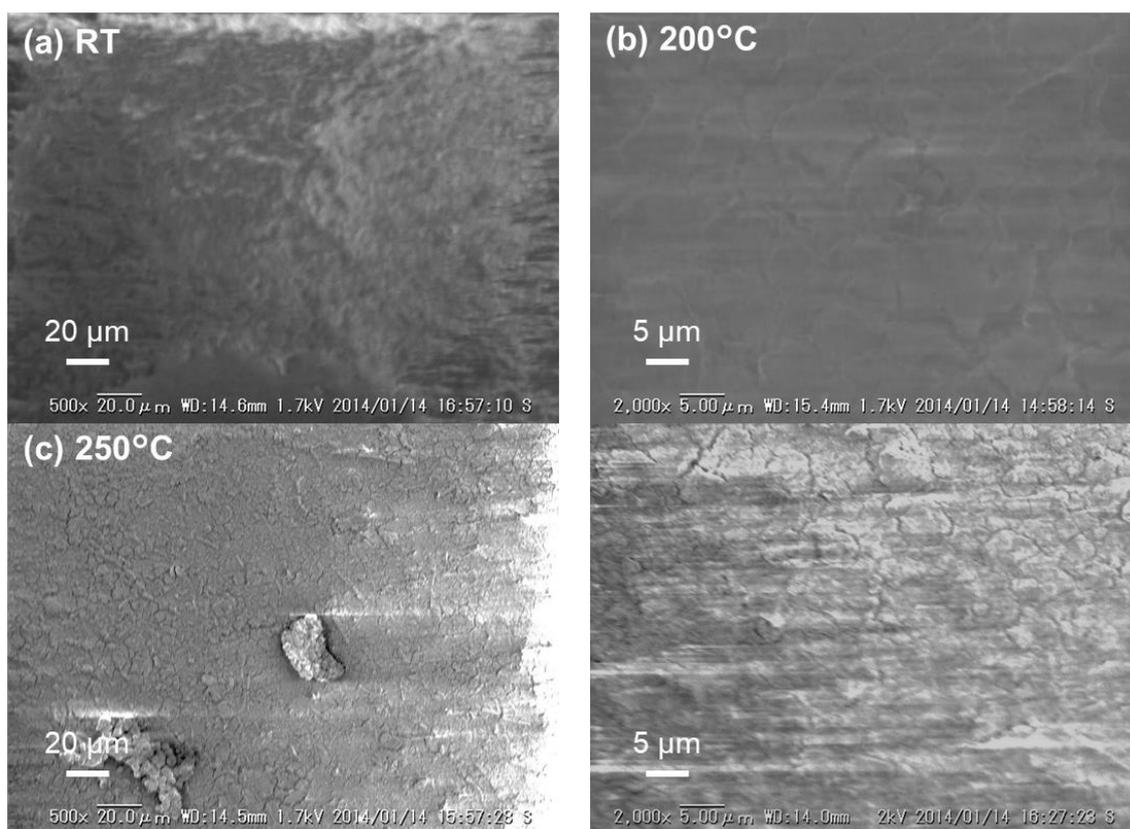


Fig. 2-14 SEM images of AAP samples after being heated in AC for 2 h at (a) 200, (b) 250, and (c) 300°C in stainless steel tubes under a gas mixture of N₂+H₂

2.4. Discussion

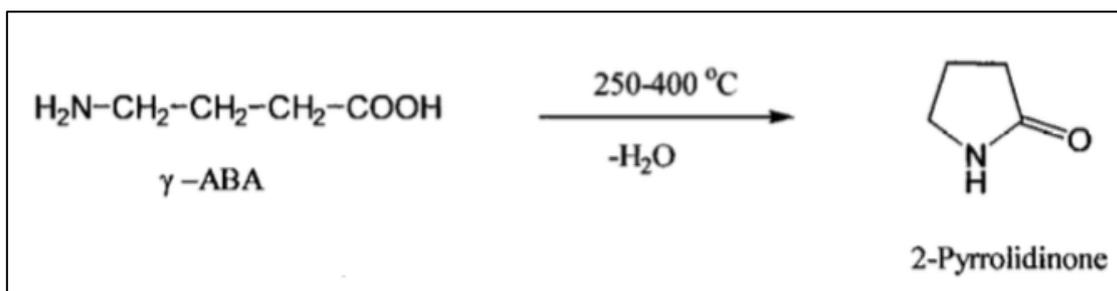
2.4.1. Stabilities of amino acids

In this experiment, I used the AAP obtained from simulating interstellar molecules and hydrothermal conditions simulating in meteorite parent bodies. In AAP, the recovery ratios of Gly and Ala heated for 2 h at 200 °C were both 40% and became 25% and 3.8%, respectively, after heating at 250 °C; further, they reduced considerably to become 0.40% and 1.3%, respectively, after heating at 300 °C. These results are consistent with the experimental results of Kurihara et al. (2012), which heated a complex organic compound synthesized from a simulating early Earth atmosphere in the simulating submarine hydrothermal system—their results showed that the recovery ratios of Gly and Ala heated for 2 h at 200 °C were 60% and 68%, respectively, whereas, at 300 °C, they became 0.4% and 3.4%, respectively. My results and Kurihara et al. (2012) both showed that amino acid concentrations decreased by heating due to the decomposition of amino acid precursor materials, and their stabilities decreased

rapidly above 200 °C. In the other words, complex organic compounds maintained their combined structure as AAP at 200 °C at least a part.

The stability of amino acids also depends on their functional groups. In this study, the concentrations of Gly, β -Ala, Asp, α - and γ -aminobutyric acid were relatively high after heating, while Ala and Ser were relatively low, indicating the differences in thermal lability among the amino acids in the AAP. Higher concentrations of Asp were observed in previous studies; for example, after heating pure seawater in a flow reactor for 20 minutes at 300 °C (Horiuchi et al. 2004) and natural samples collected from black and white smokers at temperatures >200 °C in the active hydrothermal systems (Pietrucci et al. 2018). My results and the previous studies suggested that thermal-refractory amino acids, such as Asp and γ -aminobutyric acid, remained in the form of complex macromolecular precursors even after heating at 200 °C. Also, many previous studies suggested that combining amino acids with inorganic materials in hydrothermal solutions stabilizes them (Kawahata and Ishizuka 1993; Ito et al. 2006; Chandru et al. 2013). The stability of amino acids is also controlled by their ionic form (Pietrucci et al. 2018), which is dependent on pH. Metal ions can also play a significant role in stabilizing labile amino acids via complexation (Chandru et al. 2013). Differences in the results in the metal tube from those in the glass tube (Figs. 2-7 and 2-12) might reflect the possible roles of metal ions.

The recovery ratios of γ -aminobutyric acid were considerably greater than any amino acids, and the recovery ratios reach up to >400%. It suggests not only high thermal stability of γ -aminobutyric acid but also its formation. It has been suggested that γ -aminobutyric acid is a degradation product of Glu (Cowie and Hedges 1994; Svensson et al. 2004). The stability of γ -aminobutyric acid at high temperatures was attributed to the formation of intramolecular condensates of γ -aminobutyric acid upon being heated (Scheme 1, Islam et al. 2003) and the rings were opened after acid hydrolysis. The ω -amino acids could be chemical markers of abiotic hydrothermal synthesis. Hydrothermal alteration in the amino acid composition by heating has been observed, where γ -aminobutyric acid increased with temperature increasing from 200 °C to 250 °C, and then decreased again at 300 °C.



Scheme 2-1 Formation of 2-pyrrolidinone from γ -aminobutyric acid (Islam et al. 2003)

The amino acid recovery ratios from the AAP are different from the reported values of recovery ratios of free amino acids. Kohara et al. (1997) heated an aqueous solution of amino acids at 200–350 °C for 2 h using an AC and showed that the recovery ratios of Gly and Ala heated at 200 °C were ~105% and ~95%, respectively, which then decreased after heating at 250 °C to ~85% and ~100%, respectively, and at 300 °C, the recovery ratios became ~80% and ~50%, respectively. Kobayashi et al. (1995) heated an aqueous solution of amino acids at 300 °C for 2 h using an AC and showed that the recovery ratios of Gly and Ala were 72% and 51%, respectively. The recovery ratios of Gly and Ala in their studies were much higher than those in my studies; this may be due to the higher pH in my study (pH 3.6 for Kohara et al. vs. neutral pH for my study) and/or the higher concentration of Gly and Ala in their starting material, which was 50 µM each, whereas the concentrations of Gly and Ala in the control AAP (at RT) was 30 µM and 12.8 µM, respectively. In my study, the recovery ratios of Gly and Ala at 200 °C were 40%, and the values gradually decreased to 25% and 3.8%, respectively, at 250 °C. At 300 °C, the recovery ratios of Gly and Ala showed only trace amounts, 0.4%, and 1.3%, respectively. The recovery ratios of Asp and Ser in Kohara et al.'s studies were lower than those of amino acids of this study. In this study, the recovery ratios of Asp and Ser after the AAP heated at 300°C were 60% and 10 %, respectively; however, in Kobayashi et al. (1995), the recovery ratios were 0.41% and under the detection limit, respectively, and in Kohara et al. (1997), trace amounts of Asp and Ser were recovered after 300 °C.

The amino acid recovery ratios were affected by the purged gas compositions. The recovery ratio was higher when the used pressurized gas was a mixture of nitrogen (99%) and hydrogen (1%) than when a pure nitrogen was used. Thus, it was suggested that amino acid stabilities were higher in reduced conditions. This is consistent with the results of Kohara et al. (1997) in the simulated submarine hydrothermal vent, which showed that the amino acids' recovery ratio was increased when hydrogen was added to the environmental gas.

Lastly, the AAP provided various amino acids after acid hydrolysis with high concentrations of Gly, and Ala, where they were decomposed with increasing temperature. Moreover, some amino acids released from the AAP, such as γ -aminobutyric acid, which increased with increasing temperature; this indicates that some amino acids were produced by hydrothermal alteration.

2.4.2. Alteration of the AAP

UV absorption spectra of the AAP that is treated in the AC indicated that it decomposed with increasing temperature. Results in Fig. 2-7 showed that the absorption of the AAP at 226–400 nm decreased constantly with increasing temperature, indicating that conjugated bonds such as olefinic and aromatic compounds decreased. Typically, organic compounds degraded more easily, and their stability was relatively weaker if there were fewer aromatic structures in the organic molecules. Therefore, the AAP lost its stability gradually during heating, and the molecular weight of organic matter decreased accordingly.

Generally, the heated AAP in either the Pyrex glass tube (both in N_2 and N_2+H_2) or the stainless steel tube, showed a decrease in the UV absorption at 230–400 nm with increasing temperature, indicating that organic compounds were decomposed with increasing the temperature. Decreases in the UV absorption of the AAP by heating in stainless steel tubes were less than those in Pyrex glass tubes. Metal ions from the stainless steel tube can act on the organic compounds and form complexes serving as electron donors or acceptors. Metal ions are necessary for the origin of life on the Earth as they enhance the stability of organic compounds through supporting reactions via catalytic effects and/or stabilizing organic molecules (Chandru et al. 2013; Pearson et al. 2002).

In UV fluorescence analysis, the peak intensities of the AAP generally increased with increasing the temperature. Some cyclic compounds such as aromatic and/or heterocyclic compounds were suggested to form with increasing temperature. When stainless steel tubes were used, the peak intensities were considerably lower than those when Pyrex glass tubes were used. In the case of using the Pyrex glass tube, silica could combine with organics, forming complex organic compounds, while in the case of using stainless steel tube, metal ions could combine with organics, forming complex organic compounds. Thus, the presence of silica may enhance the formation of aromatic and/or heterocyclic compounds.

In SEM analysis, aggregates were formed in the heated AAP in either the Pyrex glass tube or the stainless steel tube. These results indicated that these aggregates could be possibly formed as a result of partly alteration of the complex organics structure under hydrothermal conditions. Kurihara et al. (2012b) confirmed that aggregates could be formed in the complex organic compounds which were synthesized by proton irradiation of a gas mixture of CO, N_2 , and H_2O , the possible constituents of the primitive Earth atmosphere. These aggregates were found in the heated samples at 200 °C and over, moreover, their number was increased with increasing the heating temperature. Their results (Kurihara et al. 2012b) indicated that at 300 °C,

self-aggregates could be formed from a solution of organic compounds abiotically synthesized from the possible primitive Earth atmosphere under hydrothermal conditions. These kinds of aggregates are characterized by high tolerance against heating, and their formation might have been a significant step in the chemical evolution. Further research is needed to study the structure and functions of these aggregates and find possible formation schemes of organic aggregates in meteorite parent body environments.

2.5. Implications for meteorite parent body alteration

Primitive carbonaceous chondrites experienced aqueous alteration. Temperatures of aqueous alteration varied for the different meteorite groups and were estimated as 20–150 °C for CI, 0–80 °C for CM2, 50–150 °C for CR, and 50–340 °C for CV (Brearley 2006 and references therein). Many attempts have been made to investigate the chemical evolution of insoluble organic matter (IOM) (Quirico et al. 2014; Cody and Alexander 2005; Alexander et al. 2007, 2010; Kebukawa et al. 2011; Orthous-Daunay et al. 2013; Sephton et al. 2000, 2004) and soluble organic matter (SOM) (Martin and Sephton 2010; Glavin et al. 2010; Burton et al. 2012b; Elsila et al. 2016) in various carbonaceous chondrites during hydrothermal alteration. In this study, I investigated the hydrothermal alteration of soluble organic matter in environments simulating parent bodies of meteorites by using an AC, which is considered to be a closed hydrothermal system, especially to evaluate the effects of fluid temperatures on the recovery of amino acids, although the effects of pressure, concentration, metal ions, and pH were not well considered in the current study. As the results, the recovery ratios of Gly and Ala heated at 200 °C were up to ~40%. Considering that the alteration temperatures of CI, CM, and CR chondrites are up to 150 °C (Brearley 2006), my results are consistent with that the amino acids are abundant in non-heated carbonaceous chondrites (CI/CM/CRs) but drastically decreased in heated chondrites (e.g., CVs, COs, and heated CMs) (Elsila et al. 2016). Heating the AAP at high temperature brought the increase of γ -aminobutyric acid—this result was also consistent with the fact that relatively high abundances of ω -amino acids, including γ -aminobutyric acid, in heated chondrites (Elsila et al. 2016). In CI, CM, and CR chondrites, 38–64% of amino acids were detected without acid hydrolysis (“free” amino acids) and the rest (“bonded” amino acids) were only detectable after acid hydrolysis (Glavin et al. 2010), while only 13%–33% of total amino acids in a thermally metamorphosed CV and CO chondrites were free (except one CV with 60% free amino acids) (Burton et al. 2012a). My study indicated that a high-molecular-weight complex organic compound AAP synthesized from simulated

interstellar gas maintained their combined structure as amino acid precursors after heating at more than 200 °C. It can partly explain the high abundances of “bonded” amino acids in meteorites, particularly in heated ones.

2.6. Conclusions

Hydrothermal experiments using an autoclave (AC) were conducted with simulated interstellar complex organic compounds synthesized from carbon monoxide, ammonia, and water as an analog of complex amino acid precursors (“AAP”) to evaluate their stabilities. The AAP is known to produce various amino acids (predominantly Gly and Ala) after acid hydrolysis. Amino acids released after the heating experiments decreased with increasing temperature, but approximately 40% of Gly and Ala could survive at 200 °C. However, γ -aminobutyric acid increased after heating under a gas mixture of N_2+H_2 , indicating that γ -aminobutyric acid was synthesized during hydrothermal alteration. Finally, my data showed that the molecular structures of the AAP could be altered to form organic compounds that are more stable and can survive in elevated hydrothermal systems in meteorite parent bodies.

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Chapter 3

Synthesis of organic matter in aqueous environments simulating small bodies in the Solar System and the effects of minerals on amino acid formation

Published

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Abstract

The extraterrestrial delivery of organics to primitive Earth has been supported by many laboratory and space experiments. Minerals played an important role in the evolution of meteoritic organic matter. In this study, I simulated aqueous alteration in small bodies by using a solution mixture of H_2CO and NH_3 in the presence of water at $150\text{ }^\circ\text{C}$ under different heating durations, which produced amino acids after acid hydrolysis. Moreover, minerals were added to the previous mixture to examine their catalyzing/inhibiting impact on amino acid formation. Without minerals, glycine was the dominant amino acid obtained at 1 d of the heating experiment, while alanine and β -alanine increased significantly and became dominant after 3 to 7 d. Minerals enhanced the yield of amino acids at a short heating duration (1 d); however, they induced their decomposition at a longer heating duration (7 d). Additionally, montmorillonite enhanced amino acid production at 1 d, while olivine and serpentine enhanced production at 3 d. Molecular weight distribution in the whole of the products obtained by gel chromatography showed that minerals enhanced both the combination and decomposition of molecules. My results indicate that minerals affected the formation of amino acids in aqueous environments in small Solar System bodies and that the amino acids could have different response behaviors according to different minerals.

3.1. Introduction

Meteorites and interplanetary dust particles which came from small bodies in our Solar System, including comets and asteroids, provide valuable data on the chemical processes that occurred in the early Solar System. Extraterrestrial delivery of organic compounds including amino acids to the early Earth prior to the generation of life may have been important for the origin of life. Some carbonaceous chondrites, which include the most primitive meteorites, are mostly made up of silicates but contain abundant organic carbon up to 3–5 wt.% (Robert and Epstein 1982; Pearson et al., 2006; Alexander et al., 2007), which exists as insoluble organic matter (IOM) and soluble organic matter (SOM). Chemical records of the prebiotic synthesis reactions that occurred before and during the formation of the early Solar System and during subsequent alteration of the parent bodies are mostly provided by the SOM in carbonaceous chondrites. As some of these compounds could be precursors to biological molecules (Botta and Bada 2002; Sephton 2002; Glavin et al., 2018), the origin of these organic compounds is of interest in understanding the origin of life on Earth. In the soluble fraction of meteorites, carboxylic acids at approximately 300 parts per million (ppm) are the most abundant class of

compounds, followed by more than eighty different amino acids at approximately 60 ppm total in the Murchison CM2 carbonaceous chondrite (Sephton 2002). The early Solar System contained a wide array of organic compounds that were abiotically formed, including interstellar organics, that were incorporated into planetesimals and the parent bodies of meteorites and then finally delivered to the early Earth. By proton irradiation of a gas mixture of CO, NH₃, and H₂O, complex organic compounds could be synthesized, simulating the interstellar complex organic compounds that are known to produce amino acids after acid hydrolysis (Kobayashi et al., 1999; Takano et al., 2004; Elmasry et al., 2020). Also, a wide variety of complex organic compounds, including amino acids, can be formed through UV irradiation of simple interstellar ice mixtures and warming up prior to the accretion of planetary bodies (Bernstein et al., 2002; Muñoz Caro et al., 2002; Nuevo et al., 2008; Meinert et al., 2012; Modica et al., 2018).

Hydrothermal reactions affect both organic matter (Le Guillou et al., 2014; Alexander et al., 2017; Vinogradoff et al., 2018) and mineralogical composition (Zolensky 2005; Brearley 2006). Most of the chondrite parent bodies have experienced aqueous alteration soon after their accretion (Brearley 2006), since enough internal heating has been produced through the radioactive components (mostly ²⁶Al) (Urey 1955; Grimm and McSween 1993) to melt ice. These aqueously altered meteorites contain up to 1.3 wt.% H in water/OH mainly in the structure of phyllosilicates (Alexandar et al., 2013). The continuous interaction of water with minerals indicates progressive aqueous alteration to various degrees in carbonaceous chondrites (e.g., Zolensky et al., 1997; Brearley 2006; Trigo-Rodríguez et al., 2006; Rubin et al., 2007; Trigo-Rodríguez 2015). For example, the presence of hydrous minerals such as serpentine, which is the alteration product of olivine, indicates that aqueous alteration has occurred in the parent bodies of carbonaceous chondrites (Brearley 2006).

Chondritic insoluble organic matter was proposed to be formed through the reaction of interstellar formaldehyde followed by condensation and carbonization, possibly during a hydrothermal alteration in small bodies of the Solar System (Cody et al., 2001). Furthermore, Kebukawa et al. (2013; 2017) illustrated that the presence of ammonia significantly enhances the yield of solid organic matter from aldehydes and simultaneously produced amino acids. Various organic compounds could be also produced from formaldehyde and ammonia generated from hexamethylenetetramine degradation in environments simulating hydrothermal conditions of asteroids at 150 °C and alkaline pH for 31 days (Vinogradoff et al., 2018). Therefore, organic compounds, including amino acids, may have been synthesized from aldehydes and ammonia in aqueous environments in small bodies. Minerals could affect

hydrothermal reactions of organic matter. Rotelli et al. (2016) reported the catalytic effects of minerals of carbonaceous chondrites in the presence of water and formamide in the production of a diverse range of organic compounds (nucleobases, amino acids, and carboxylic acids) that are important for the origin of life and confirmed that some reactive minerals could have served as catalysts to promote increasing organic complexity in chemical evolution. It is suggested that phyllosilicates may have acted as absorbents and catalysts in the early Solar System for the organic precursor of molecule reactions (Pearson et al., 2002) since the key mechanisms involved are their surface reactions and interlayer space reactivity, which can explain their function as absorbent or catalyst (Schoonen et al., 2004; Ganor et al., 2009). The presence of phyllosilicates could catalyze the organic compounds to be more abundant and more complex, and the nature of the phyllosilicate impacted the abundance and the molecular composition of the final organic compounds, including amino acids (Vinogradoff et al., 2020a; 2020b). The different chemical composition of the phyllosilicates suggests different oxidation states, as well as the release of different cations in solution by dissolution, possibly leading to the formation of various complexes rich in organic matter, and, in turn, different catalyzing/inhibiting reactions (Vinogradoff et al., 2020a). Yamashita and Naraoka (2014) demonstrated that the presence of olivine powder promoted the formation of alkylpyridines from aldehydes and ammonia (the Chichibabin synthesis). Overall, the presence and the nature of the silicates could affect the abundance and molecular composition of the final organic compounds in primitive bodies during hydrothermal alteration. In the current research, I evaluated the effect of minerals, namely, olivine, montmorillonite, and serpentine for amino acid production in conditions simulating water-bearing parent bodies.

3.2. Methods

3.2.1. Samples and hydrothermal experiments

The organic compounds were synthesized using a starting solution (200 μL) containing 77.3 μL of 36.0-38.0% (13 M) formaldehyde aqueous solution, 8.4 μL of 25.0-27.9% (14 M) ammonia aqueous solution, and water (145 μL), at a molar ratio of $\text{H}_2\text{CO}:\text{NH}_3:\text{H}_2\text{O} = 9:1:100$ (5 M H_2CO and 0.6 M NH_3). I refer to the product as “FAW” after formaldehyde–ammonia–water. The experiments were conducted following the method of the amino acid synthesis experiments of Kebukawa et al. (2017), but without glycolaldehyde and $\text{Ca}(\text{OH})_2$. To evaluate the effect of minerals in chondritic meteorites on amino acid production, I added minerals (10 g/L) to the FAW solution mixture. The minerals used were olivine ($(\text{Mg}, \text{Fe})_2\text{SiO}_4$) from San

Carlos, Arizona, USA, Na-montmorillonite $((\text{Na,Ca})_{0.33}(\text{Al, Mg, Fe})_2\text{Si}_4\text{O}_{10}(\text{OH})_2 \cdot n\text{H}_2\text{O})$ —“SWy-1” from the Clay Minerals Society, and serpentine (antigorite) $((\text{Mg,Fe})_3\text{Si}_2\text{O}_5(\text{OH})_4)$ from Miyatsu, Kyoto, Japan, purchased from Nichika Corp. #14-4-12-1. The mineral powders were washed with hexane before use, and then fully dried at room temperature (RT). Each solution was flame-sealed into glass tubes after O_2 elimination from the system using a vacuum after cooling with liquid nitrogen, and then these tubes were heated at $150\text{ }^\circ\text{C}$ over reaction periods of 1 d, 3 d, or 7 d in an oven (ETTAS HTO-300S). Each set of conditions was repeated three or more times. Control samples were prepared using a starting solution contained formaldehyde and water (without ammonia) at a molar ratio of $\text{H}_2\text{CO}:\text{H}_2\text{O} = 9:100$. Minerals (10 g/L) were added to the solution mixture under the same experimental conditions ($150\text{ }^\circ\text{C}$, at 1 d, 3 d, or 7 d). Unheated samples (0 day samples) were also prepared using formaldehyde, ammonia, and water at a molar ratio of $\text{H}_2\text{CO}:\text{NH}_3:\text{H}_2\text{O} = 9:1:100$ as a starting solution. In addition, unheated samples with minerals (10 g/L) were prepared. Both control and unheated samples were analyzed with the same procedures. All the glassware used was baked at $500\text{ }^\circ\text{C}$ for at least three hours prior to use in all experiments and the Millipore Milli-Q[®] system was used to purify water used in this synthetic experiment and the subsequent analytical procedures.

3.2.2. High-Performance Liquid Chromatograph (HPLC) Analysis

After heating, an aliquot (150 μL) of the FAW was mixed with 150 μL of 6 M HCl for acid hydrolysis and heated at $110\text{ }^\circ\text{C}$ for 24 h to convert them into their corresponding amino acids. Most of the amino acids are stable under the previous classical conditions of hydrolysis and can be calculated quantitatively (Davidson 2003; Rutherford and Gilani 2009). The FAW was dried by vacuum centrifugation at $60\text{ }^\circ\text{C}$ after acid hydrolysis. The hydrolyzed fraction underwent desalting with a cation-exchange resin (AG 50W-X8 resin). The resin (5 mL) was sequentially prewashed with the following liquids: 1 M HCl (20 mL), water (20 mL), 1 M NaOH (20 mL), water (20 mL), 1 M HCl (20 mL), and water (20 mL). After filtration with polytetrafluoroethylene (PTFE) membrane filters (DISMIC-13HP 0.45 μm) and rinsing with 0.1 M HCl (1 mL) and water (20 mL), an aliquot of 0.1 M HCl sample solution (1 mL) was inserted into the column. Then, the amino acids-containing solution was eluted successively with 10% NH_3 aqueous solution (15 mL) and water (20 mL). The $\text{NH}_3 + \text{H}_2\text{O}$ elute was centrifuged dried at $60\text{ }^\circ\text{C}$, and the dried sample was fully dissolved in 1 M NaOH (0.5 mL) and 1 mL of water, then dried again by vacuum centrifugation at $60\text{ }^\circ\text{C}$. Finally, the dried sample was dissolved by adding 0.1 M HCl (40 μL) and 160 μL of water. The control and

unheated samples were treated under the same previous preparation protocol (acid hydrolysis followed by desalting).

A 1- μ L aliquot of each FAW solution with and without minerals was filtrated through cellulose acetate membrane filters (DISMIC-3CP 0.45 μ m) and analyzed by using an Ultra-High-Performance Liquid Chromatograph (UHPLC, Nexera X2, Shimadzu, Kyoto, Japan) system equipped with an autosampler (SIL-30AC). To separate the amino acids, a reversed-phase column (Inertsil-ODS4 column, 100 mm L. \times 3.0 mm I.D.) was used. The UHPLC system was equipped with an LC-30AD solvent delivery unit, RF-20Axs fluorescence detector, CBM-20A/lite system controller, and CTO-20AC column oven which maintains the temperature at 35 °C. Chromatography was performed by gradient elution with mobile phases of (A) a solution of 15 mmol/L potassium dihydrogen phosphate and 5 mmol/L dipotassium hydrogen phosphate, and (B) acetonitrile:methanol:water = 45:40:15 (v/v/v) at a flow rate of 0.8 mL/min. Fluorescence detection used excitation at 350 nm and emission at 450 nm. The derivatization reagents used were 0.1 mol/L borate buffer (pH 9.2) solutions of mercaptopropionic acid (MPA) (a mixture of 10 mL borate buffer and 10 μ L MPA) and o-phthalaldehyde (OPA) (a mixture of 0.7 mL borate buffer, 10 mg OPA, 0.3 mL ethanol, and 4 mL water), acetonitrile solution of chloroformic acid 9-fluorenylmethyl (FMOC) (a mixture of 25 mL acetonitrile and 10 mg FMOC), and 0.1 mol/L potassium phosphate buffer (a mixture of 0.34 mL 85% phosphoric acid and 0.68 g potassium dihydrogen-phosphate) (pH 2). For peak identification, commercial standard solutions of the amino acid (Wako Amino Acids Mixture Standard Solution, Type B and Type AN-2) were used. In the control experiments (without ammonia under the same experimental conditions), only trace amounts of amino acids were detected after acid hydrolysis. The concentrations (μ M) of amino acids are control-corrected values that were obtained by subtraction of the control samples from the amino acid quantities of the FAW. The error bars reflect experimental uncertainties based on the repeated heating experiments under the same protocol.

3.2.3. FTIR micro-spectroscopy analysis

Fourier transform infrared (FTIR) micro-spectroscopy was performed to evaluate the evolution of the molecular structure of the organic compounds derived from the FAW solution that occurred due to the heating period and mineral addition. After hydrothermal experiments, the soluble fraction was separated through centrifugation, and then one droplet was placed on a CaF₂ plate (13 mm diameter \times 1 mm thickness), and dried. IR absorption spectra were obtained through a Fourier-transform infrared micro-spectrometer (micro-FTIR; FT/IR-

6100typeA+IRT-5200, JASCO), equipped with a mercury-cadmium-telluride (MCT) detector, a ceramic IR light source, a germanium-coated KBr beam splitter, and $\times 16$ Cassegrain mirrors. The microscope and the FTIR were continually purged with dry N_2 during analysis. A total of 128 scans of IR transmission spectra were accumulated in the wavenumber range of 8000–400 cm^{-1} , with a wavenumber resolution of 4 cm^{-1} , with a 20 $\mu m \times 20 \mu m$ aperture. Background spectra were obtained via blank areas of the CaF_2 plates adjacent to the samples. Several different points (3–5) were analyzed for each sample, and the required peak ratios were calculated for each spectrum. All the peak ratios were then averaged for each sample.

3.2.4. Gel filtration chromatography analysis

Gel filtration chromatography is a chromatographic technique in which molecules are separated by their molecular weight and was used to evaluate the changes in the molecular weight of FAW samples in response to the heating duration and mineral addition. After the hydrothermal experiments, all FAW samples were filtrated through PTFE membrane filters (DISMIC-13HP 0.45 μm). A Shimadzu HPLC system was used, which was equipped with an SPD-M20A photodiode detector, two LC-20AD pumps, DGU-20A5 degassing unit, and a CTO-20AC column oven that provides precise temperature control at 30 $^{\circ}C$. The absorbance detection wavelength was at 260 nm and the sample injection amount was 5 μL . Chromatography was performed using a mobile phase of 0.3 M ammonium hydrogen carbonate buffer (pH = 8.0–8.1) at a flow rate of 0.5 mL/min. Protein standards with known molecular weights composed of lactalbumin (14,073 Da), r-insulin (5807 Da), and vitamin B12 (1355 Da) were used. The molecular weight of the collected samples could be estimated from a graph of the logarithm of the molecular weight as a function of the retention time of the protein standards.

3.3. Results

3.3.1. Amino acid concentrations

After acid hydrolysis, the amino acids concentrations of the heated mixture of formaldehyde and ammonia in the presence of water (FAW) with and without minerals were quantified using UHPLC. Figure 3-1 displays typical chromatograms of the unheated FAW with and without minerals, treated at RT (0 day samples), and the heated FAW with and without minerals, heated at 150 $^{\circ}C$ for 1 d, 3 d, and 7 d, comparing the addition of different minerals. To understand the relative peak sizes, the y-scale and x-scale used for all chromatograms in Figure 3-1 are the same. Amino acids were identified by comparing their retention time with

those in the known standards (Fig. 3-2). After acid hydrolysis, all FAW mixtures with and without minerals including the unheated FAW treated at RT (0 d) and those heated at different heating periods (1 d, 3 d, and 7 d) exhibited a wide range of amino acids including glycine (Gly), alanine (Ala), β -alanine (β -Ala), serine (Ser), aspartic acid (Asp), glutamic acid (Glu), and γ -aminobutyric acid (γ -ABA). The concentrations (μM) of the amino acids (Table 3-1, Figs. 3-2–3-5) are calculated from control-corrected values of three or more separate measurements that were obtained by subtraction of the control samples (without ammonia under the same experimental conditions) from the amino acid quantities of the FAW. Although the γ -ABA and some other peaks appeared clearly in some samples, I could not discuss them, since their concentrations showed unexplained errors. For all the unheated FAW (0 d) treated at RT, the total amino acids concentrations were only small amounts with and without minerals. The total amino acids concentrations for FAW without minerals, with olivine, with montmorillonite, and with serpentine were 3.8 ± 2.0 , 2.6 ± 2.2 , 2.8 ± 0.8 , and 5.8 ± 1.1 μM , respectively. Gly was the dominant amino acid in all unheated FAW samples and its concentrations for FAW without minerals, with olivine, with montmorillonite, and with serpentine were 1.6 ± 1.0 , 1.3 ± 1.1 , 2.0 ± 0.4 , and 2.4 ± 0.2 μM , respectively.

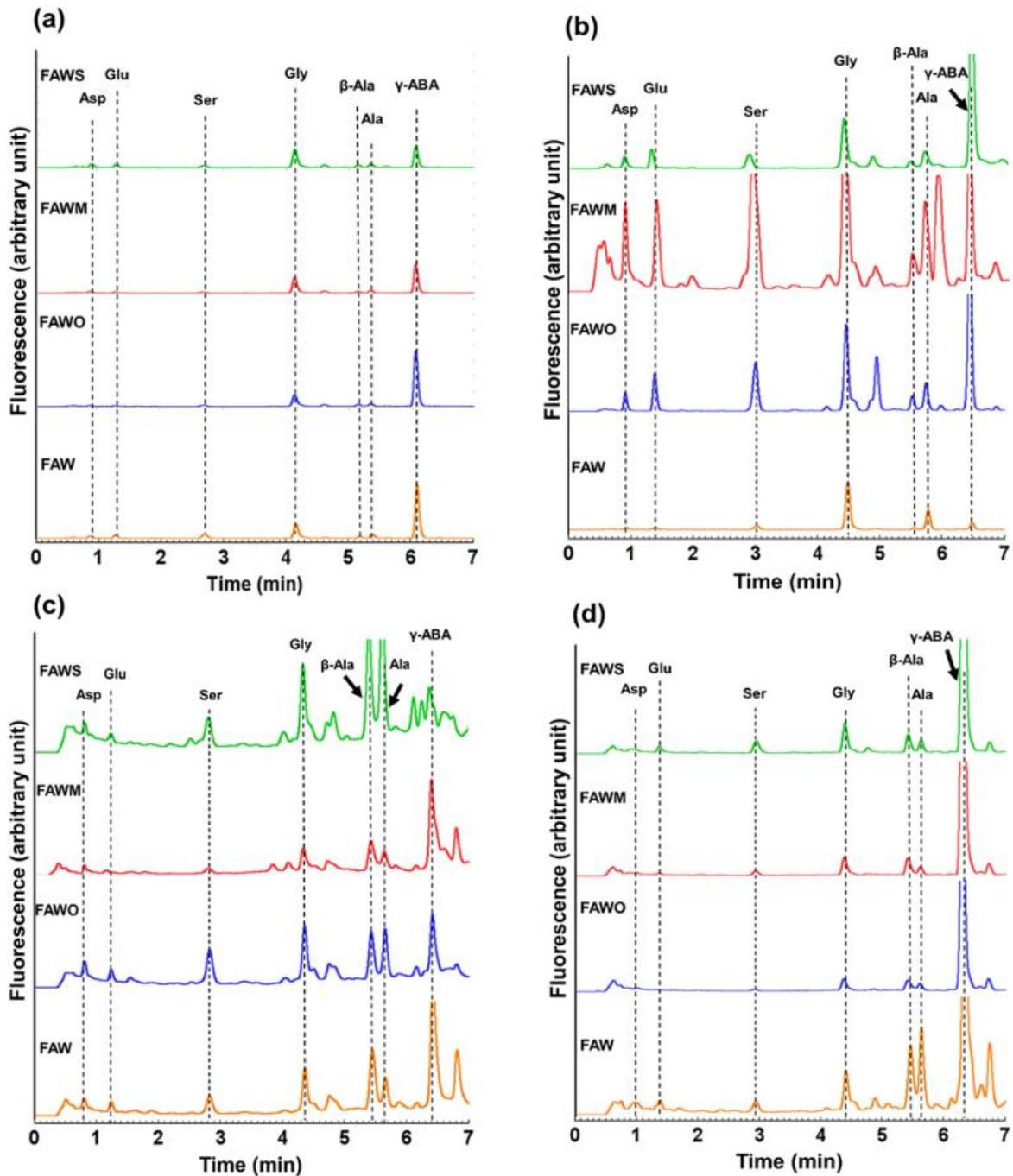


Fig. 3-1 Chromatograms of the acid-hydrolyzed (a) unheated formaldehyde-ammonia-water (FAW) treated at RT (0 d), and FAW heated at 150 °C for (b) 1 d, (c) 3 d, and (d) 7 d under the following conditions: without minerals “FAW”, with added olivine “FAWO”, with added montmorillonite “FAWM”, and with added serpentine “FAWS”. Asp: aspartic acid, Glu: glutamic acid, Ser: serine, Gly: glycine, Ala: alanine, β -Ala: β -alanine, γ -ABA: γ -aminobutyric acid.

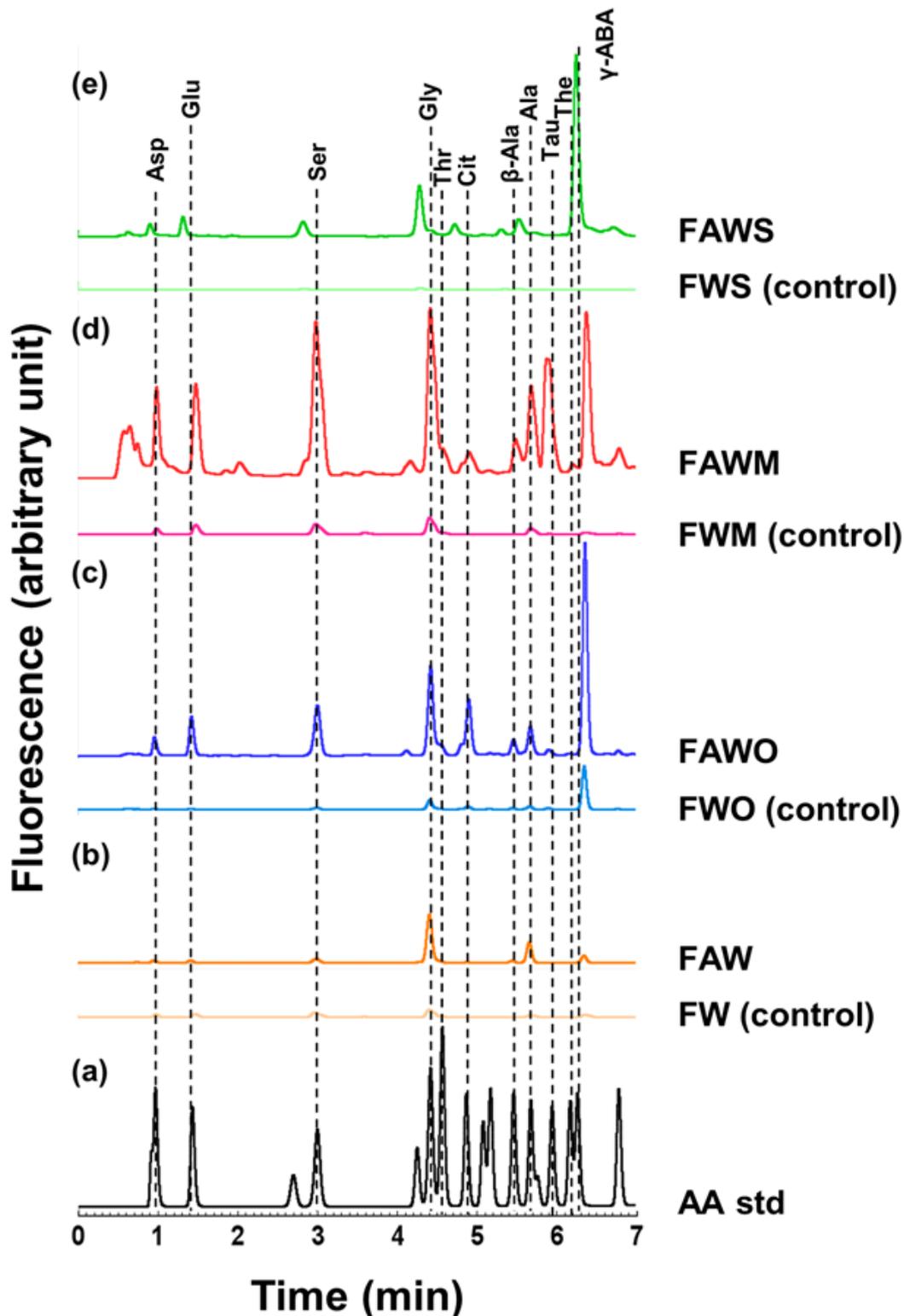


Fig. 3-2 Chromatograms of (a) the amino acid standard, and the acid-hydrolyzed samples with and without ammonia (control) heated at 150 °C for 1 d under the following conditions: (b) without minerals “FAW”, (c) with added olivine “FAWO”, (d) with added montmorillonite “FAWM”, and (e) with added serpentine “FAWS”. Asp: aspartic acid, Glu: glutamic acid, Ser: serine, Gly: glycine, Thr: threonine, Cit: citruline, β-Ala: β-alanine, Ala: alanine, Tau: taurine, The: theanine, γ-ABA: γ-aminobutyric acid.

Table 3-1. A summary of the average control-corrected amino acid concentrations in the FAW after acid hydrolysis. FAW: without minerals, FAWO: with olivine, FAWM: with montmorillonite, and FAWS: with serpentine.

Amino Acid concentration (μM)	FAW				FAWO			
	RT	150°C			RT	150°C		
	0 d	1 d	3 d	7 d	0 d	1 d	3 d	7 d
Gly	1.6 ± 1.0	8.7 ± 7.5	3.4 ± 1.7	9.5 ± 2.2	1.3 ± 1.1	9.4 ± 3.7	7.2 ± 3.6	6.6 ± 5.7
Ala	0.3 ± 0.1	3.3 ± 1.0	4.3 ± 1.9	34.3 ± 9.6	0.3 ± 0.2	7.5 ± 3.2	6.4 ± 1.2	8.9 ± 7.0
β -Ala	0.4 ± 0.3	1.4 ± 1.1	5.9 ± 3.0	17.5 ± 5.4	0.1 ± 0.08	4.5 ± 1.9	5.6 ± 1.2	6.0 ± 1.9
Ser	0.6 ± 0.2	2.0 ± 1.5	0.6 ± 0.2	6.2 ± 2.1	0.4 ± 0.3	10.4 ± 6.5	3.7 ± 0.6	9.7 ± 12.2
Asp	0.5 ± 0.3	1.9 ± 0.9	2.4 ± 1.1	4.3 ± 1.3	0.24 ± 0.2	5.7 ± 2.7	3.8 ± 0.4	4.8 ± 3.3
Glu	0.4 ± 0.2	2.2 ± 0.4	0.9 ± 0.5	4.0 ± 1.6	0.3 ± 0.2	7.7 ± 2.7	4.5 ± 1.1	1.7 ± 1.9
Total	3.8 ± 2.0	19.5 ± 10.5	17.5 ± 4.9	75.8 ± 9.1	2.6 ± 2.2	45.2 ± 15.8	31.2 ± 5.8	37.7 ± 30.8

Amino Acid concentration (μM)	FAWM				FAWS			
	RT	150°C			RT	150°C		
	0 d	1 d	3 d	7 d	0 d	1 d	3 d	7 d
Gly	2.0 ± 0.4	19.6 ± 12.0	1.3 ± 0.7	3.5 ± 0.9	2.4 ± 0.2	18.0 ± 2.1	6.8 ± 5.8	7.3 ± 2.2
Ala	0.2 ± 0.1	22.4 ± 9.4	2.3 ± 0.9	2.4 ± 0.2	1.0 ± 0.4	5.4 ± 1.4	21.5 ± 4.2	4.3 ± 0.9
β -Ala	0.2 ± 0.1	9.0 ± 2.7	4.8 ± 1.5	6.7 ± 0.7	0.4 ± 0.02	3.7 ± 2.3	15.3 ± 1.7	8.7 ± 3.2
Ser	0.1 ± 0.06	22.6 ± 13.8	0.4 ± 0.3	3.1 ± 1.1	0.6 ± 0.4	6.0 ± 2.3	1.6 ± 0.6	5.5 ± 0.8
Asp	0.1 ± 0.04	12.0 ± 4.3	2.0 ± 0.3	2.0 ± 0.6	0.6 ± 0.04	4.3 ± 0.8	6.9 ± 1.3	3.0 ± 1.2
Glu	0.2 ± 0.1	20.0 ± 11.6	1.3 ± 0.7	0.6 ± 0.03	0.8 ± 0.1	8.1 ± 4.3	3.6 ± 0.8	2.2 ± 1.9
Total	2.8 ± 0.8	105.6 ± 18.6	12.1 ± 3.3	18.3 ± 1.6	5.8 ± 1.1	45.5 ± 5.2	55.7 ± 5.1	31.0 ± 8.5

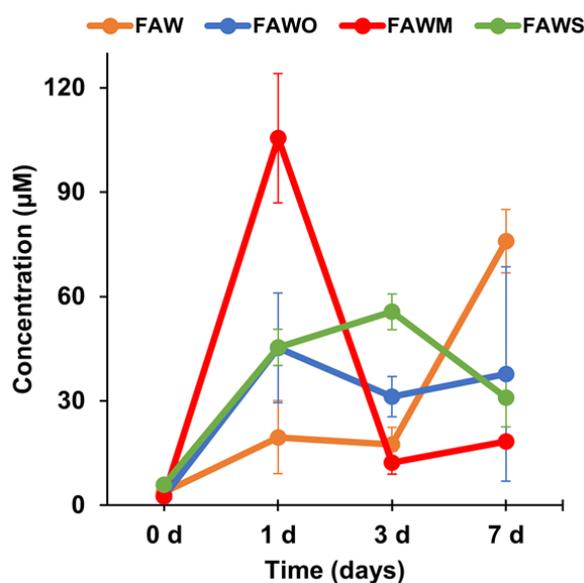


Fig. 3-3 Total amino acid concentrations (control-corrected values) of the unheated FAW samples treated at RT (0 d) and the heated FAW at 150 °C for 1 d, 3 d, and 7 d after acid hydrolysis. Note that the error is the standard deviation (1σ) of three or more separate experimental runs. The total amino acids include six amino acids: Asp, Glu, Ser, Gly, β -Ala, and Ala.

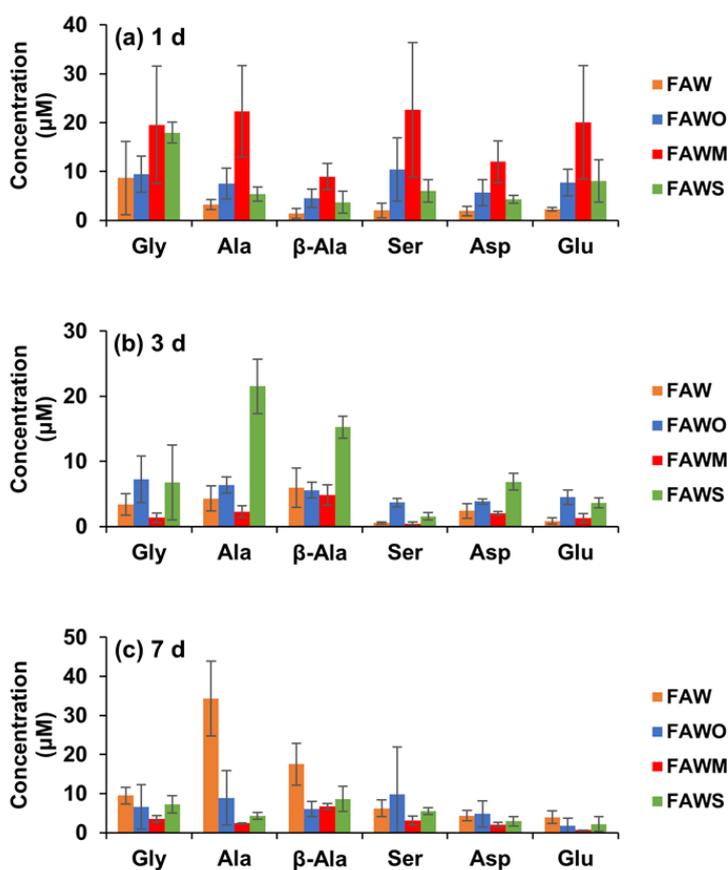


Fig. 3-4 Amino acid concentrations (control-corrected values) in the FAW samples (with and without minerals) heated at 150 °C after acid hydrolysis for (a) 1 d, (b) 3 d, and (c) 7 d. The error is the standard deviation (1σ) of three or more separate experimental runs.

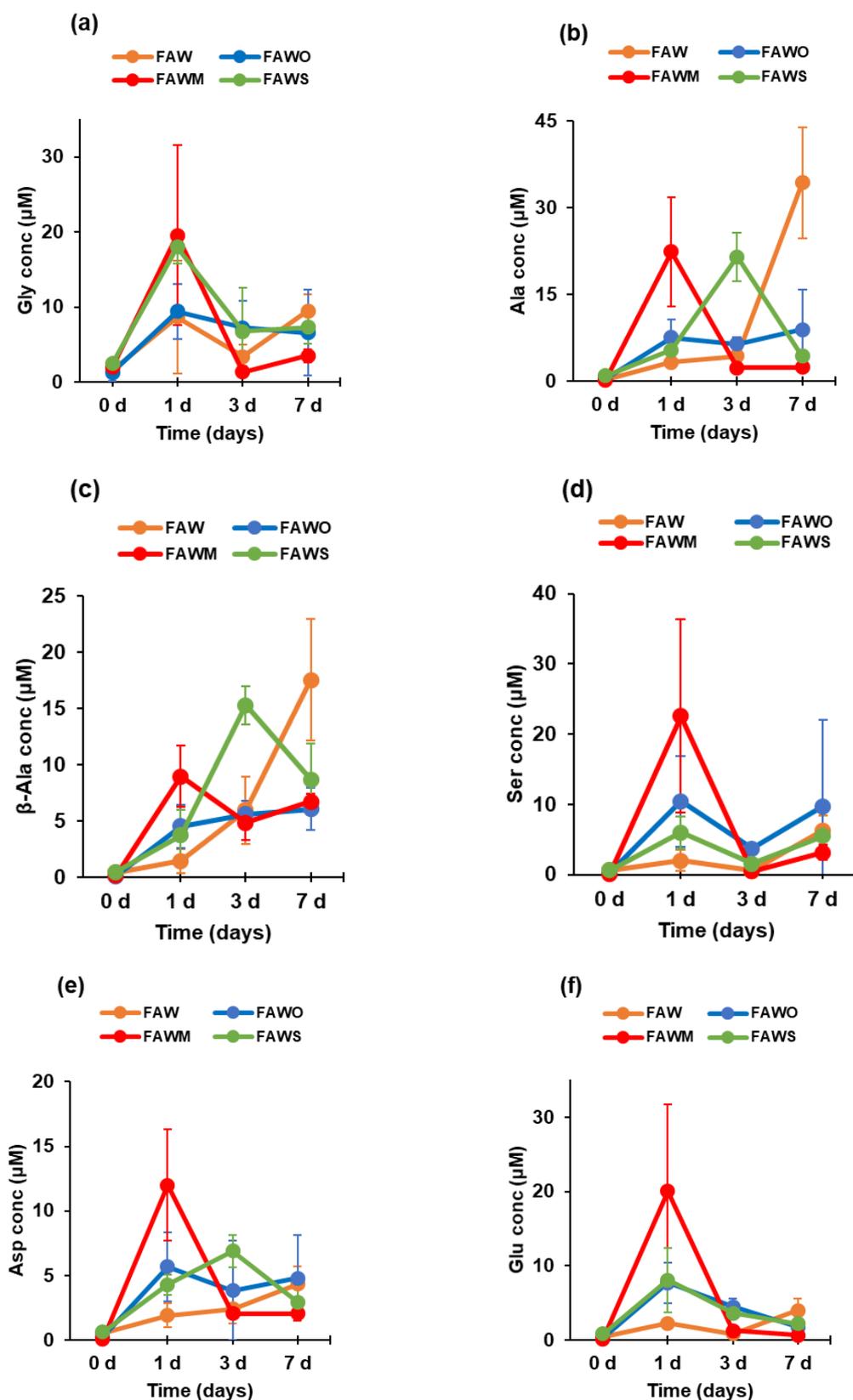


Fig. 3-5 Amino acid concentrations (control-corrected values) of the unheated FAW treated at RT (0 d) and the FAW samples heated at 150 °C for 1 d, 3 d, and 7 d after acid hydrolysis. The error is the standard deviation (1σ) of three or more separate experimental runs.

Figure 3-3 presents the change in total amino acid concentrations with time depending on mineral addition after acid hydrolysis. After heating at 150°C, the concentration of the amino acids increased significantly for all samples with and without minerals. For FAW without minerals, the total amino acid concentration after heating for 1 d and 3 d were 19.5 ± 10.5 and 17.4 ± 4.9 μM , respectively, and then increased to 75.9 ± 9.1 μM for 7 d. However, these results changed in response to the addition of minerals, and the concentrations of the amino acids have different response behaviors according to different minerals. For the 1 d experiment, the total amino acids concentrations were almost the same in the presence of olivine and serpentine at around 40 μM . After increasing the heating duration from 1 d to 7 d, the total amino acid yields were almost constant at around 40 μM . Therefore, it seems that olivine and serpentine had almost the same effect on the total amino acid formation. As a consequence, the total amino acids with these minerals were higher than the case without minerals at 1 d and 3 d, yet lower than without minerals at 7 d. Montmorillonite had a different effect on amino acid production, since the amino acids production at 1 d was the highest among all tested samples, whereas the amino acid concentrations decreased sharply after heating for 3 d and 7 d to the lowest observed concentration. Overall, montmorillonite enhanced amino acid production after heating for 1 d, while olivine and serpentine enhanced production for 3 d. After 7 d, all minerals had a negative effect on the production of the amino acids.

Figures 3-4 and 3-5 show each amino acid concentration (after acid hydrolysis) in the heated FAW samples. In FAW without minerals, the Gly concentrations for 1–7 d were almost stable with time within the margin of error, despite the slight changes—the concentration of Gly heated for 1 d was 8.7 ± 7.5 μM and became 3.4 ± 1.7 μM , after heating for 3 d. Its concentration increased again to 9.5 ± 2.2 μM after heating for 7 d (Fig. 3-5a). The rest of the amino acids showed a relative increase, especially Ala and β -Ala, which increased notably after heating for 7 d. The concentrations of Ala and β -ala heated for 1 d were 3.3 ± 1.0 μM and 1.4 ± 1.1 μM , respectively, increasing to 4.3 ± 1.9 μM and 5.9 ± 3.0 μM , respectively, after 3 d heating; then the concentrations increased significantly after 7 d heating, increasing to 34.3 ± 9.6 μM and 17.5 ± 5.4 μM , respectively (Fig. 3-5b,c). The dominant amino acid in 1 d heated FAW without minerals was Gly; however, β -Ala and Ala became the most abundant amino acids in the FAW heated for 3 d and 7 d (Fig. 3-4). My results for the amino acid production from hydrothermal experiments conducted for 3 d without mineral addition revealed that β -Ala was the most abundant amino acid, followed by Ala then Gly, even though their concentrations seemed to be very close. However, the experimental results of Kebukawa et al. (2017) (experimental details are provided in the discussion section) from hydrothermal experiments

performed at 150 °C for 3 d revealed that Ala was the most dominant, followed by Gly then β -Ala.

After adding olivine, the four amino acids, Gly, Ala, β -Ala, and Asp were produced at 3 d and 7 d with similar amounts to those at 1 d heating, in contrast to Glu, which continuously decreased at 3 d and 7 d (Fig. 3-5). All amino acid concentrations were the highest with montmorillonite among the cases with other minerals and without minerals at 1 d, and α -amino acids were dominant (Fig. 3-4a). Subsequently, all amino acids decreased drastically after 3 d and 7 d (Fig. 3-5), and Ala and β -Ala became the dominant amino acid in some cases at 3 d and 7 d (Fig. 3-4b,c). With serpentine, the dominant amino acid at 1 d was Gly, followed by Ala and β -Ala, which became dominant after 3 d; the concentrations of these amino acids decreased after 7 d and β -Ala and Gly became dominant (Fig. 3-4). Ser and Asp were relatively stable with time (Fig. 3-5d,e), while Gly and Glu decreased gradually with time (Fig. 3-5a,f). It should be noted that Gly decreased with time, but even Gly became dominant at 7 d with the addition of serpentine (all amino acids decreased at 7 d). Ala, β -Ala, and Asp yields were enhanced by serpentine at 3 d, then decreased again after 7 d (Fig. 3-5b,c,e).

3.3.2. FTIR absorption spectra

FTIR was used to track the changes in the bulk molecular structures of the heated mixtures of formaldehyde and ammonia solutions (FAW) at different heating durations with various mineral additions. Various functional groups were detected in the IR absorption spectra of dried solutions of heated FAW (Figs. 3-6–3-8) and the identified peak assignments are summarized in Table 3-2. The heated FAW exhibited absorption bands (1) in the region of 3685–3000 cm^{-1} , characteristic of OH stretching modes including carboxyl, alcohol, and associated water, (2) at 2960-2970 and 2935-2940 cm^{-1} due to CH_3 asymmetric stretching and CH_2 asymmetric stretching, respectively, and at the 2885 cm^{-1} band containing both CH_3 and CH_2 symmetric stretching, since the symmetric stretching bands of CH_2 at 2870–2840 cm^{-1} and CH_3 at 2885–2865 cm^{-1} cannot be clearly separated, (3) at approximately 1760, approximately 1700, and 1600 cm^{-1} due to C=O (ester), C=O (carboxyl, aldehyde, and amide), and aromatic C=C bands, respectively, and (4) at 1050 cm^{-1} due to C–O stretching modes (Socrates 2004). It should be noted that no peaks from silicates were observed since mineral phases (solid phases) were separated from the FAW solutions by centrifuging.

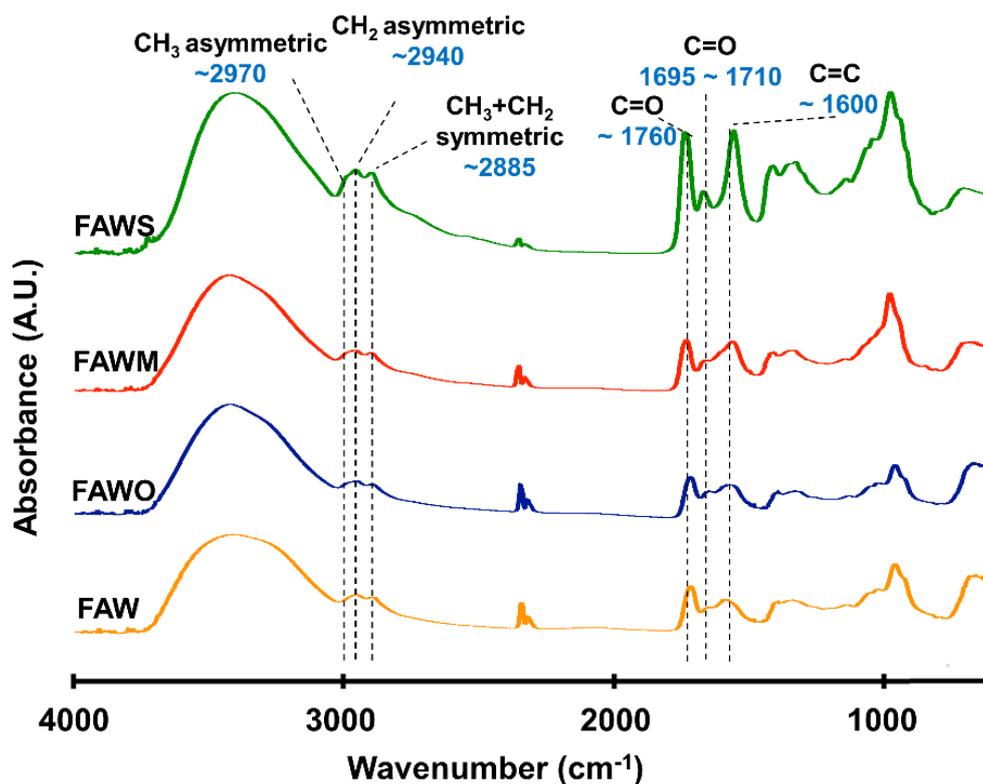


Fig. 3-6 Micro-FTIR spectra for the region of 700–4000 cm^{-1} of FAW heated at 150 $^{\circ}\text{C}$ for 1 d.

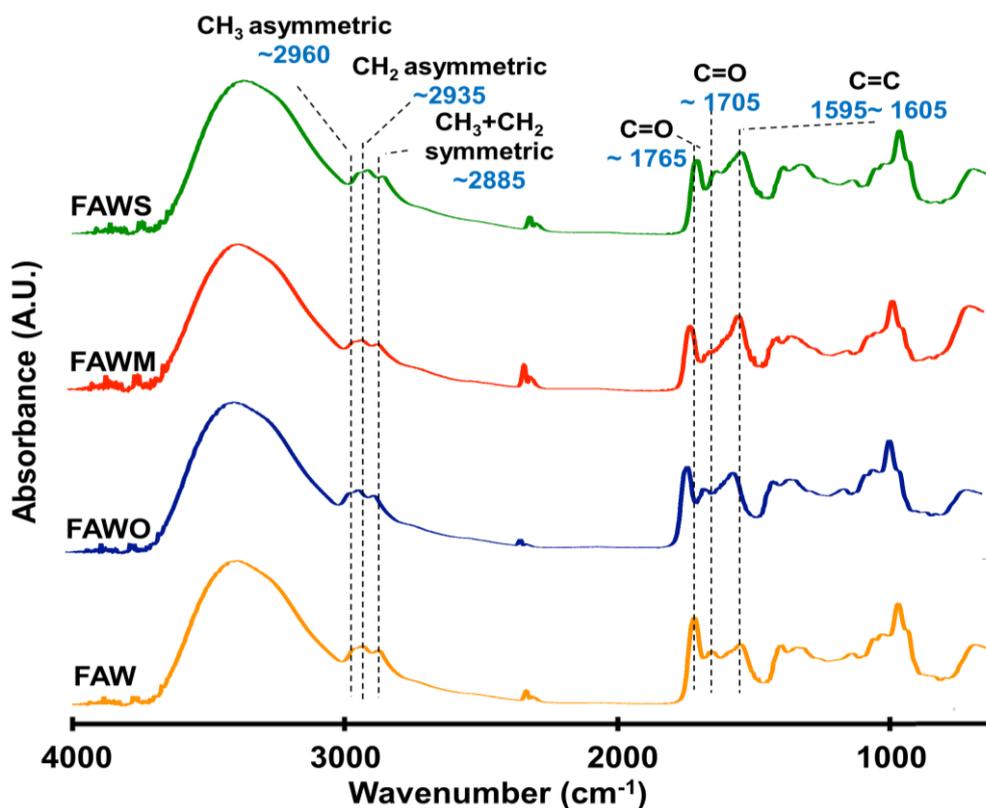


Fig. 3-7 Micro-FTIR spectra for the region of 700–4000 cm^{-1} of FAW heated at 150 $^{\circ}\text{C}$ for 3 d.

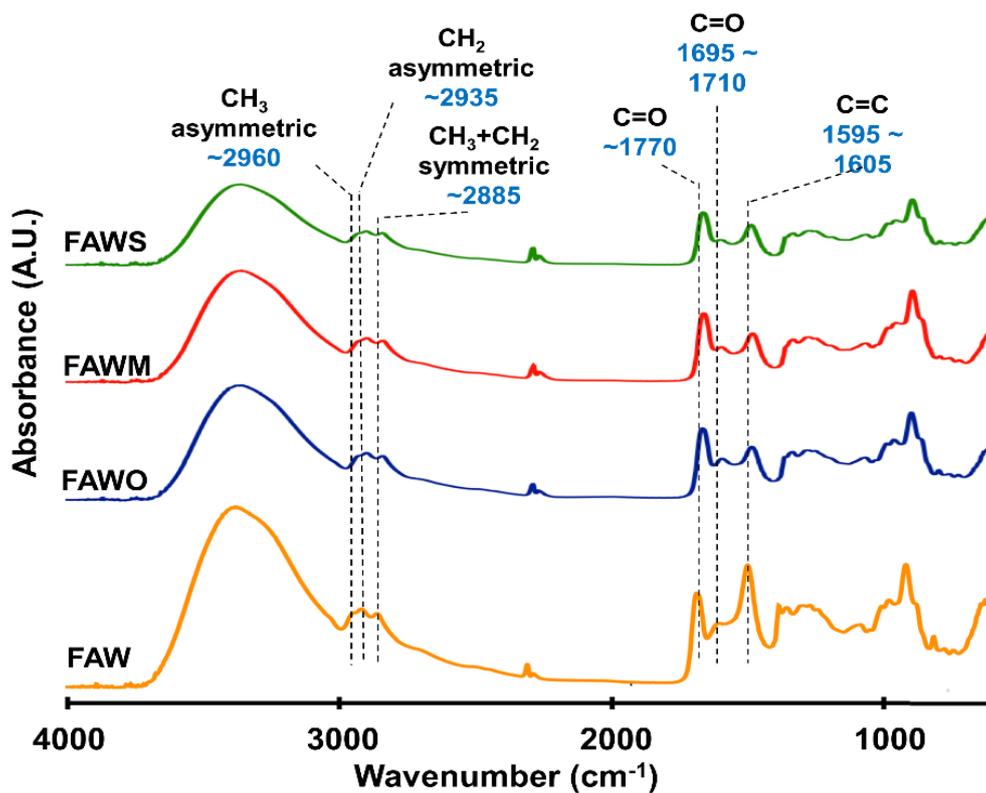


Fig. 3-8 Micro-FTIR spectra for the region of 700–4000 cm^{-1} of FAW heated at 150 $^{\circ}\text{C}$ for 7 d.

Table 3-2. Summary of IR peak assignments of FAW based on Socrates (Socrates 2004).

Peak Position (Wavenumber/ cm^{-1})	Assignments	Species
3370	OH	Carbonyl, alcohol, associated water
2960-2970	C–H asymmetric stretch	Aliphatic CH_3
2935-2940	C–H asymmetric stretch	Aliphatic CH_2
2885	C–H symmetric stretch	Aliphatic CH_3+CH_2
1760-1770	C=O stretch	Ester
1695-1710	C=O stretch	Carboxyl, aldehyde, amide
1595-1605	C=C stretch	Olefinic, aromatic
1050	C–O stretch	

FTIR absorption spectra without ammonia

Figures 3-9 to 3-11 show the IR spectra of the liquid phase (dried) of the heated FAW and the sample solutions without ammonia under the same experimental conditions as control for amino acid concentrations. There were some differences in the functional groups between the IR spectra with and without ammonia. For example, peaks at around 1770 cm^{-1} due to the C=O functional group in FAW without ammonia were not detected. Additionally, the peak heights of aliphatic C-H were markedly higher without ammonia than with ammonia.

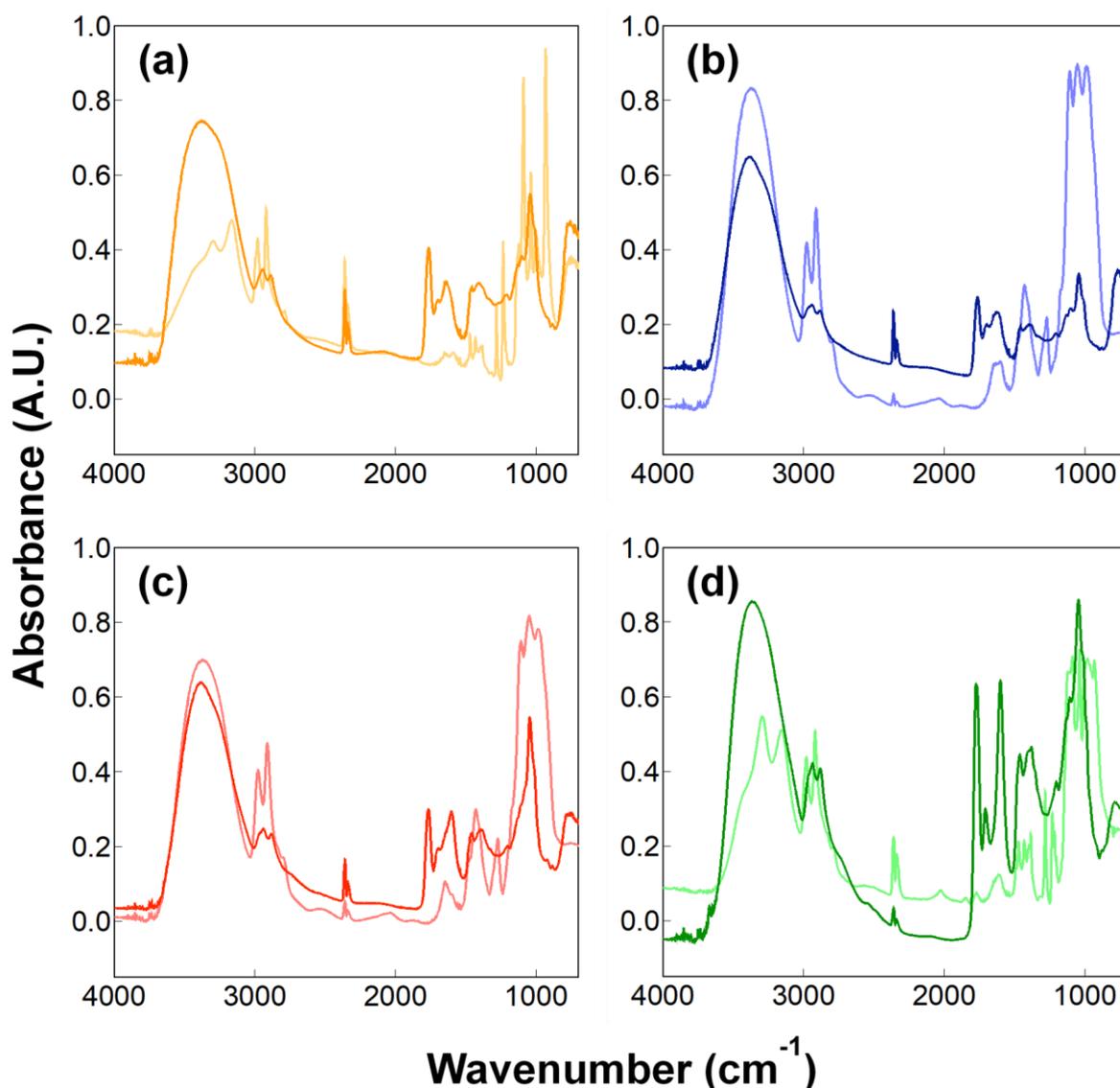


Fig. 3-9 Micro-FTIR spectra for the region of $700\text{--}4000\text{ cm}^{-1}$ of the solution (with and without ammonia) heated at $150\text{ }^{\circ}\text{C}$ for 1 d under the following conditions: (a) without minerals, (b) with olivine, (c) with montmorillonite, and (d) with serpentine. The faint line is without ammonia.

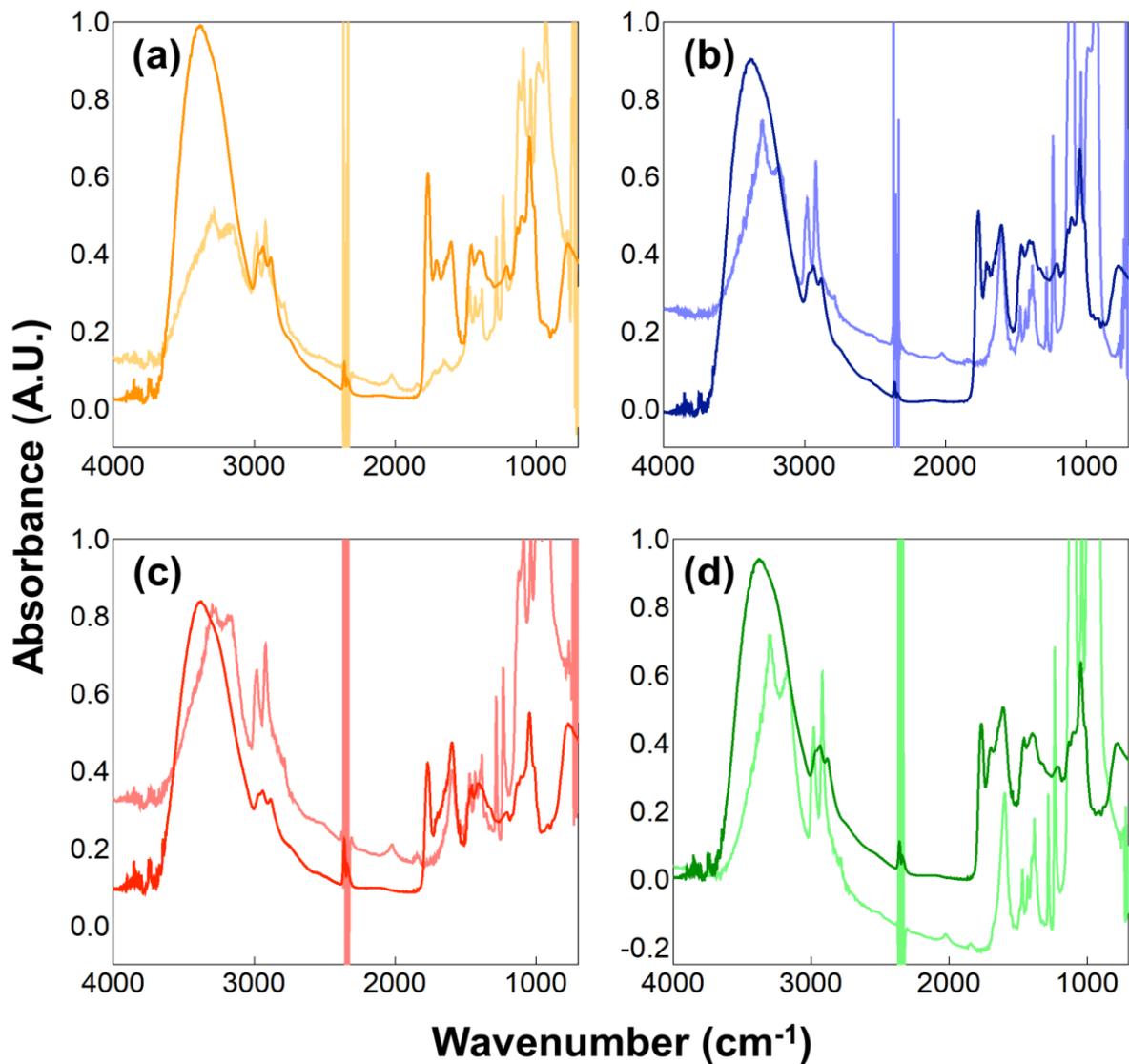


Fig. 3-10 Micro-FTIR spectra for the region of 700–4000 cm^{-1} of the solution (with and without ammonia) heated at 150 °C for 3 d under the following conditions: (a) without minerals, (b) with olivine, (c) with montmorillonite, and (d) with serpentine. The faint line is without ammonia.

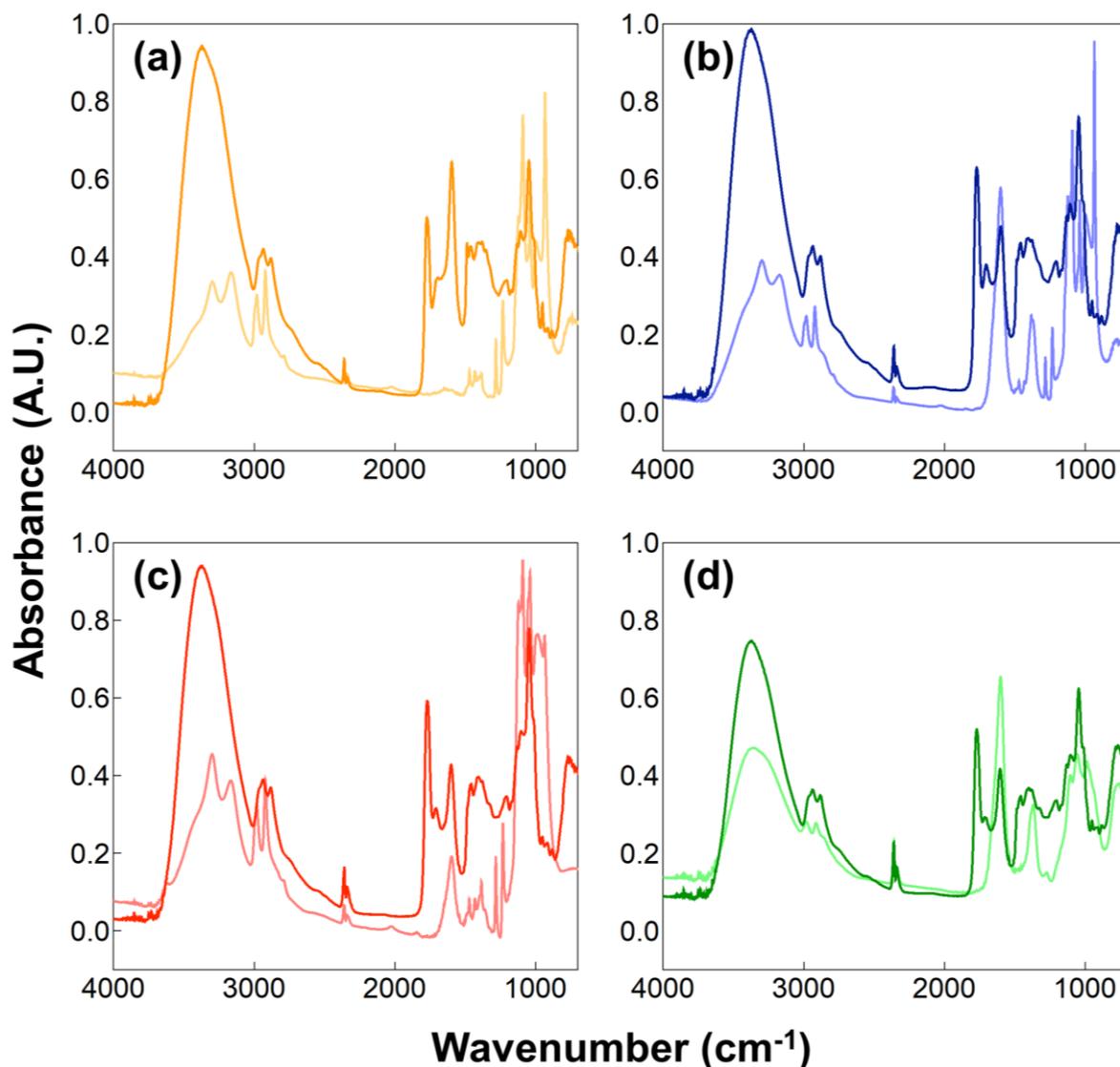


Fig. 3-11 Micro-FTIR spectra for the region of 700–4000 cm^{-1} of the solution (with and without ammonia) heated at 150 $^{\circ}\text{C}$ for 7 d under the following conditions: (a) without minerals, (b) with olivine, (c) with montmorillonite, and (d) with serpentine. The faint line is without ammonia.

The IR absorption spectra of heated FAW showed a relative change due to mineral addition as well as increasing heating duration. The reproducibility was confirmed, since micro-FTIR spectra were obtained from several different areas for each sample, and the average of the peak height ratios and the standard deviation of the mean (1σ) was calculated (see Section 3.2.1, Fig. 3-12, and Table 3-3)—the error could be due to the heterogeneity of the sample. There were some changes in the peak positions of functional groups under the effect of increasing heating durations and adding minerals (Table 3-4). For instance, aliphatic CH_3 and CH_2 peaks were equally shifted to lower wavenumbers, from 2970 cm^{-1} and 2945 cm^{-1} , respectively, down to 2960 cm^{-1} and 2935 cm^{-1} , respectively, under the effect of time and minerals. The

C=O stretch peak shifted from 1760 cm^{-1} (the ester region) to higher wavenumbers (1770 cm^{-1}) under the effect of minerals and time. It should be noted that the peak positions of the C=C stretching bands around 1600 cm^{-1} decreased from 1645 cm^{-1} to 1595 cm^{-1} with time in FAW without minerals. In FAW with olivine, the C=C stretch peak position decreased from 1620 cm^{-1} to 1600 cm^{-1} , while the addition of phyllosilicates (montmorillonite and serpentine) did not show a significant difference in the peak position with time. The C=C peak position changed notably after adding minerals at 1 d of heating.

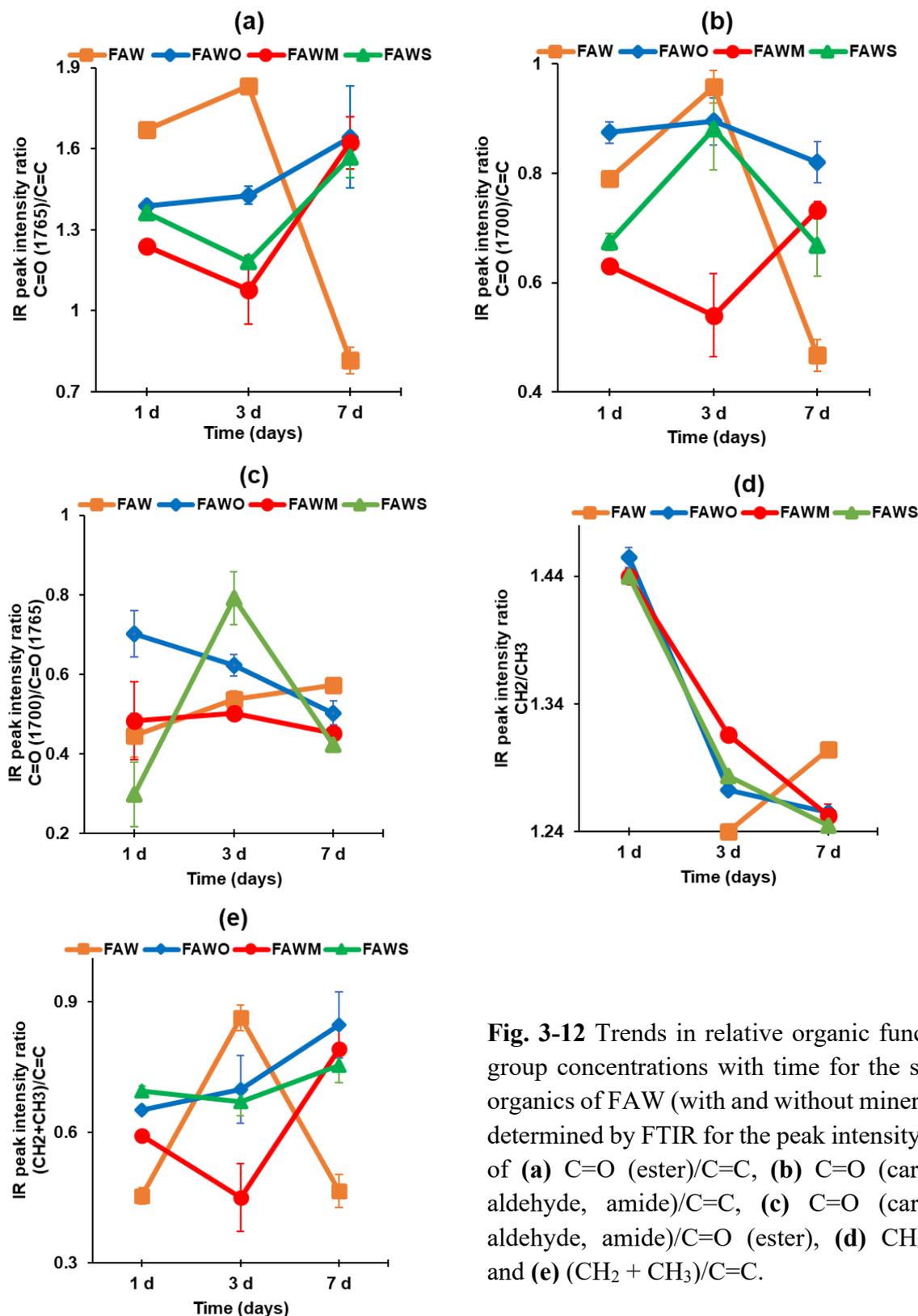


Fig. 3-12 Trends in relative organic functional group concentrations with time for the soluble organics of FAW (with and without minerals) as determined by FTIR for the peak intensity ratios of (a) C=O (ester)/C=C, (b) C=O (carboxyl, aldehyde, amide)/C=C, (c) C=O (carboxyl, aldehyde, amide)/C=O (ester), (d) CH₂/CH₃, and (e) (CH₂ + CH₃)/C=C.

The C=O (ester), C=O (carboxyl, aldehyde, amide), and C=C peak intensities are obtained by the peak top height of the band around 1765, 1700, and 1600 cm⁻¹, respectively, with the linear baseline between 1840 and 1520 cm⁻¹. The CH₂ and CH₃ peak intensities are obtained by the peak top height of the band at 2935 cm⁻¹ (CH₂) and the band at 2960 cm⁻¹ (CH₃), respectively, with the linear baseline between 3010 and 2790 cm⁻¹.

Table 3-3. IR peak intensity ratios. C=O and C=C peak intensities are obtained by the peak top height of the band around 1765 (ester), 1700 (carboxyl, aldehyde, amide), and 1600 cm^{-1} (C=C), respectively, with the linear baseline between 1840 and 1520 cm^{-1} . The CH_2 and CH_3 peak intensities are obtained by the peak top height of the band at 2935 cm^{-1} (CH_2) and the band at 2960 cm^{-1} (CH_3), respectively, with the linear baseline between 3010 and 2790 cm^{-1} .

C=O (1765)/ C=C	1 d	3 d	7 d	C=O (1700)/ C=C	1 d	3 d	7 d
FAW	1.67 ± 0.02	1.83 ± 0.01	0.82 ± 0.05	FAW	0.79 ± 0.003	0.96 ± 0.03	0.47 ± 0.03
FAWO	1.39 ± 0.01	1.43 ± 0.03	1.64 ± 0.19	FAWO	0.87 ± 0.02	0.89 ± 0.04	0.82 ± 0.04
FAWM	1.24 ± 0.01	1.08 ± 0.13	1.62 ± 0.10	FAWM	0.63 ± 0.01	0.54 ± 0.08	0.73 ± 0.02
FAWS	1.36 ± 0.02	1.18 ± 0.02	1.57 ± 0.07	FAWS	0.67 ± 0.02	0.88 ± 0.08	0.67 ± 0.01
C=O (1700)/ C=O (1765)	1 d	3 d	7 d	CH_2/CH_3	1 d	3 d	7 d
FAW	0.45 ± 0.06	0.54 ± 0.02	0.57 ± 0.01	FAW		1.24 ± 0.002	1.30 ± 0.004
FAWO	0.70 ± 0.06	0.62 ± 0.03	0.50 ± 0.03	FAWO	1.45 ± 0.01	1.27 ± 0.003	1.26 ± 0.01
FAWM	0.48 ± 0.10	0.50 ± 0.01	0.45 ± 0.01	FAWM	1.44 ± 0.01	1.32 ± 0.003	1.25 ± 0.01
FAWS	0.30 ± 0.08	0.79 ± 0.07	0.42 ± 0.01	FAWS	1.44 ± 0.002	1.28 ± 0.002	1.24 ± 0.003
(CH_2+CH_3)/ C=C	1 d	3 d	7 d				
FAW	0.45 ± 0.02	0.86 ± 0.03	0.47 ± 0.04				
FAWO	0.65 ± 0.01	0.70 ± 0.08	0.85 ± 0.07				
FAWM	0.59 ± 0.002	0.45 ± 0.08	0.79 ± 0.05				
FAWS	0.69 ± 0.01	0.67 ± 0.03	0.75 ± 0.04				

Table 3-4. Summary of functional group peak positions of the FAW.

Peak Position (Wavenumber/ cm^{-1})	FAW			FAWO			FAWM			FAWS		
	1 d	3 d	7 d	1 d	3 d	7 d	1 d	3 d	7 d	1 d	3 d	7 d
CH_3	-*	2960	2960	2970	2960	2960	2970	2960	2960	2970	2960	2960
CH_2	2945	2938	2935	2940	2935	2935	2940	2940	2935	2935	2935	2935
C=O (ester)	1760	1765	1770	1760	1765	1770	1765	1768	1770	1770	1665	1770
C=O (Carboxyl, aldehyde, amide)	1700	1705	1695	1698	1705	1705	1695	1700	1710	1710	1695	1710
C=C	1645	1603	1595	1620	1603	1600	1600	1595	1600	1600	1605	1605

* CH_3 peak could not be separated for FAW 1 d.

IR peak intensity ratios

Since the peaks of C=O at approximately 1765 cm^{-1} and 1700 cm^{-1} , and C=C at approximately 1600 cm^{-1} were clearly distinguished (Figs 3-6–3-8), the peak height ratios were obtained with a linear baseline correction between 1840 and 1520 cm^{-1} . Figure 3-12a shows peak height ratios of $\sim 1765\text{ cm}^{-1}$ (C=O in ester) over $\sim 1600\text{ cm}^{-1}$ (C=C). This ratio exhibited an increase in value with time in the presence of minerals and decreased with time without minerals. Figure 3-12b shows the peak height ratios of $\sim 1700\text{ cm}^{-1}$ (C=O in carboxyl, aldehyde, and amide) over $\sim 1600\text{ cm}^{-1}$ (C=C). This ratio exhibited a relative variation with time and the addition of minerals, since it decreased with time in FAW samples except in FAW with montmorillonite, and decreased after heating for 3 d, yet finally increased after heating for 7 d. The peak height ratio of $\sim 1700\text{ cm}^{-1}$ (C=O in carboxyl, aldehyde, and amide) and $\sim 1765\text{ cm}^{-1}$ (C=O in ester) were also plotted (Fig. 3-12c). This ratio exhibited a relative decrease with time in the presence of minerals; however, it increased in FAW without minerals.

Figure 3-12e shows the total aliphatic/aromatic ($(\text{CH}_2+\text{CH}_3)/\text{C}=\text{C}$) intensity ratios, with higher values with added minerals than for the heated FAW without minerals at 1 d, and this ratio keeps increasing with time. At the same time, FAW without added minerals showed an increase in this ratio at 3 d, which then essentially decreased at 7 d. CH_2/CH_3 intensity ratios were obtained from the peak heights of the aliphatic CH_3 asymmetric stretching ($\sim 2960\text{ cm}^{-1}$) and CH_2 asymmetric stretching ($\sim 2935\text{ cm}^{-1}$) bands (Fig. 3-12d). This ratio decreased with time in FAW with minerals; however, in the case of FAW without mineral addition, the ratio increased (from 3 d to 7 d; CH_2 could not be separated in case of 1 d). My results with mineral addition are consistent with those of Kebukawa and Cody (Kebukawa and Cody 2015), showing that the CH_2/CH_3 ratios decreased with time in the solid organic products synthesized from formaldehyde, glycolaldehyde, ammonia, and water in the presence of $\text{Ca}(\text{OH})_2$. It should be noted that the peak height ratio is proportional to the relative amounts of CH_3 and CH_2 groups in the heated FAW samples. The aliphatic CH_2/CH_3 ratio approximately represents the length of the aliphatic chain length and the levels of branching (Igisu et al., 2009); the higher ratio indicates longer aliphatic chains, and the lower ratio indicates shorter chain lengths or higher branching levels.

3.3.3. Molecular weight estimation

Figures 3-13 to 3-15 present gel filtration chromatograms of the heated FAW with and without minerals heated at $150\text{ }^\circ\text{C}$ for 1 d, 3 d, and 7 d, respectively. It should note that the

original chromatograms of the FAW heated for 7 d were delayed by 3.5 min due to the instrumental conditions, and the delay was corrected. Three major peaks denoted as Peak 1, 2, and 3, could be distinguished in all chromatograms at retention times of 14.4, 16.5, and 18.0 min, respectively. In my experiments, the shape of the detected peaks was approximately the same in all chromatograms. Therefore, I only considered these three major peaks for further interpretation. Peaks eluted at lower retention times corresponded to high molecular weight compounds, while the lower molecular weight compounds eluted at higher retention times. It is suggested that molecules eluted before 20 min have high molecular weights of at least several hundred Da or a few thousand Da. Chromatograms of the protein standard and a logarithm graph of its molecular weight as a function of retention time are also shown in Figure 3-16. If I assume that FAW behaves like proteins in the gel filtration column, the molecular weights of Peak 1–3 can be estimated as approximately 7930–7380, 5430–5370, and 4990–4960 Da, respectively. However, it should be noted that these molecular weight calculations are uncertain, and it is just an estimation based on the results with the protein molecular weight standards. Thus, my discussion is based on the relative changes in the molecular weight distributions with mineral additions.

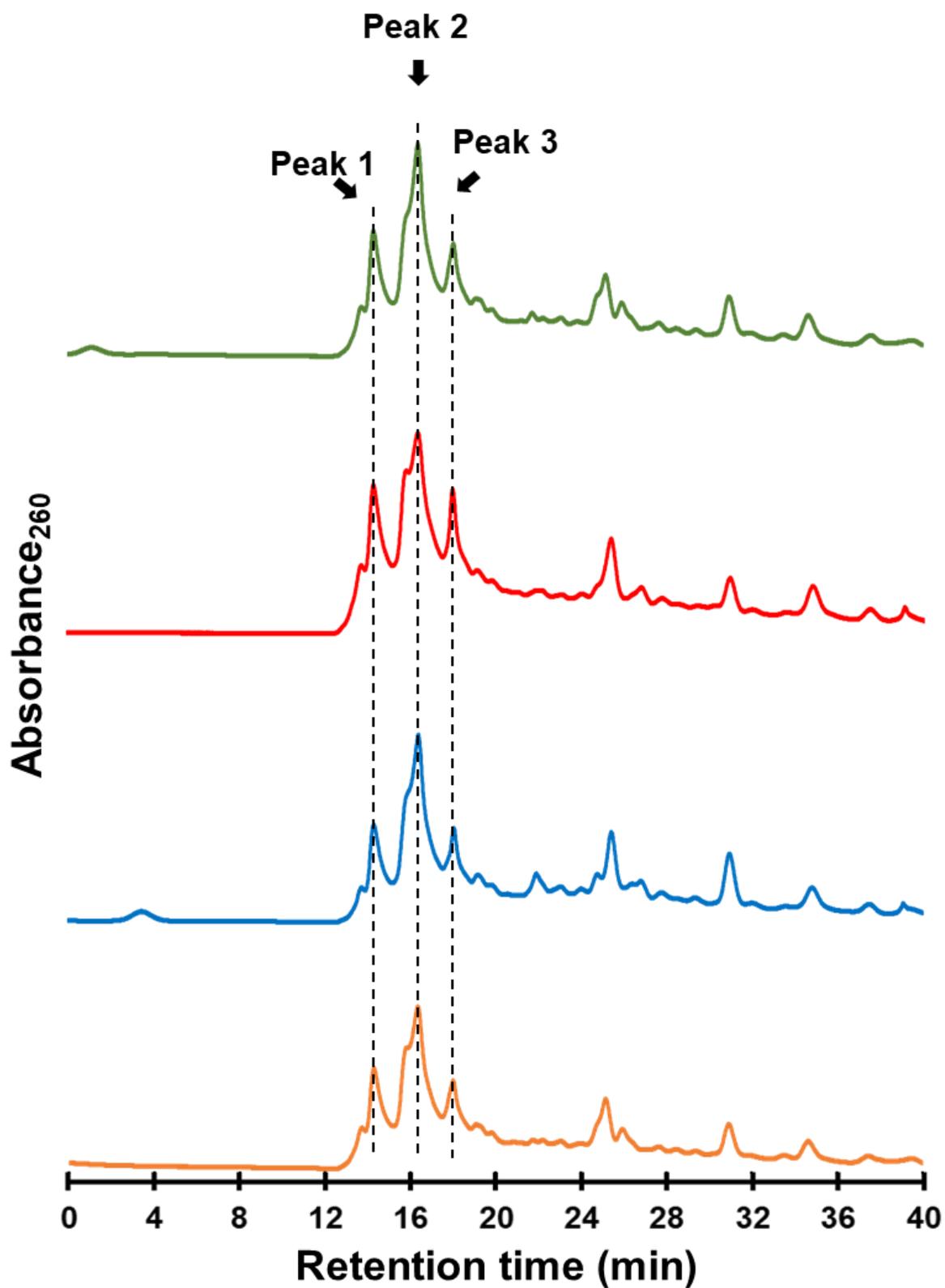


Fig. 3-13 Gel filtration chromatograms of the FAW heated at 150 °C for 1 d.

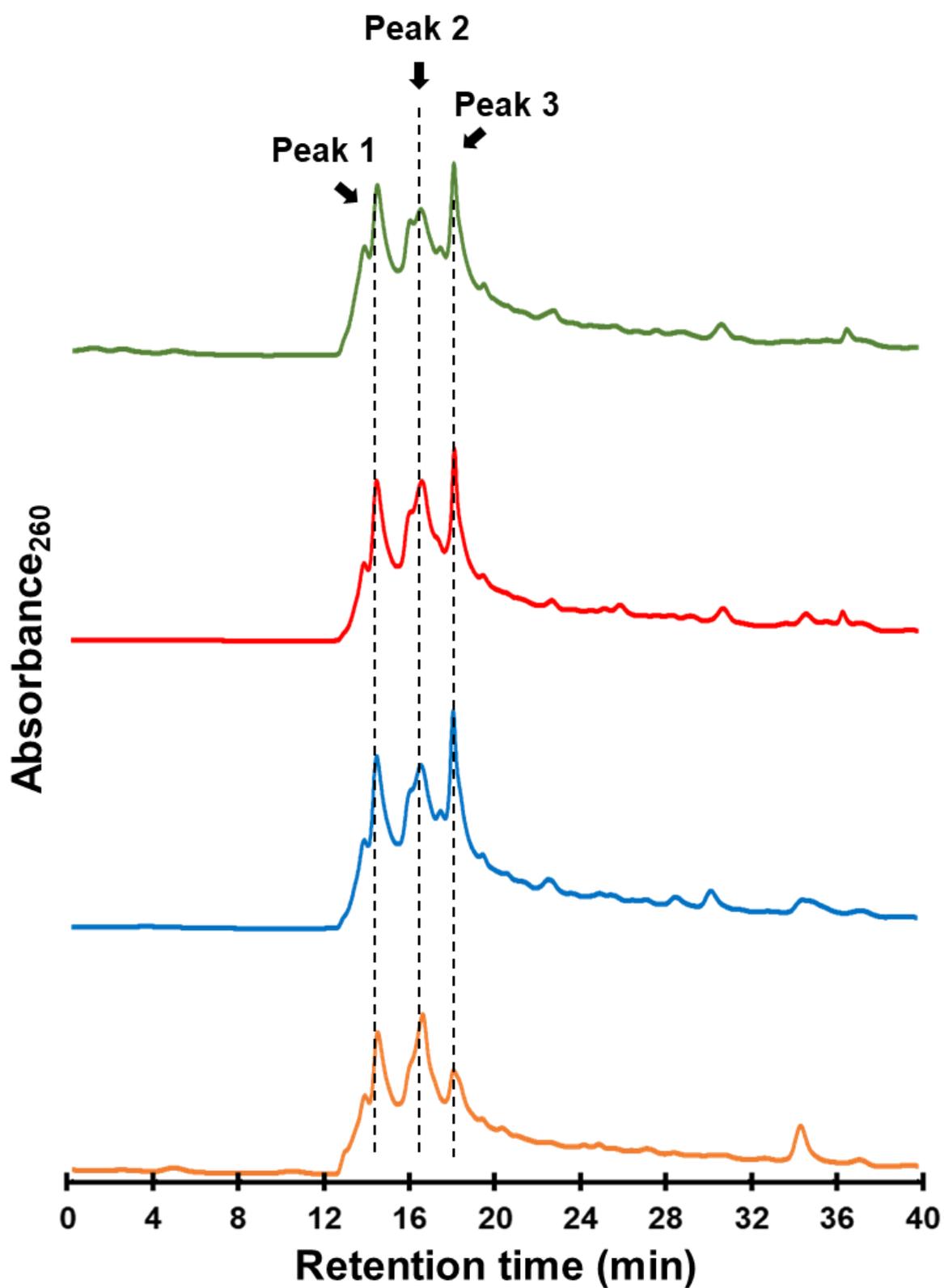


Fig. 3-14 Gel filtration chromatograms of the FAW heated at 150 °C for 3 d.

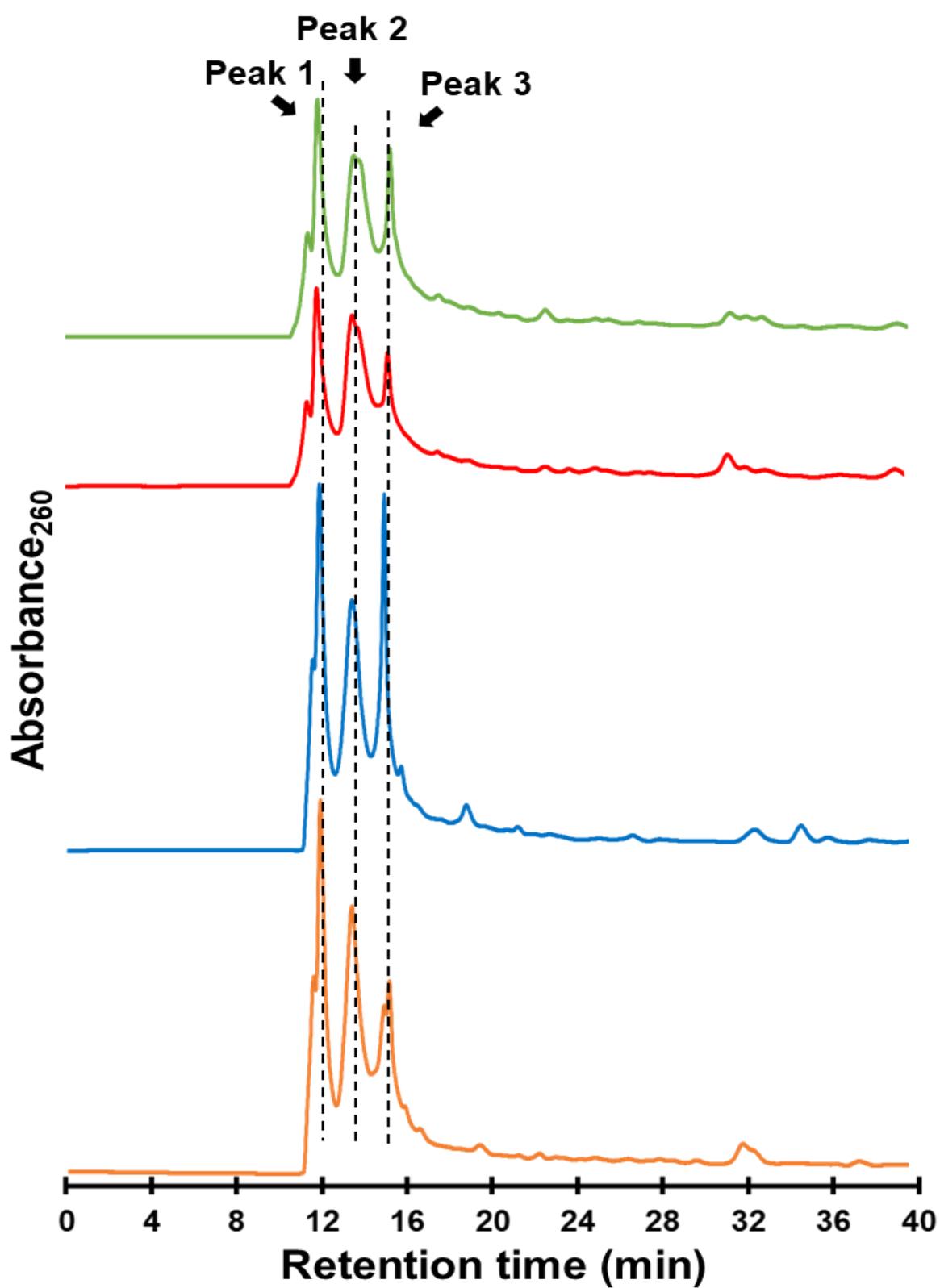


Fig. 3-15 Gel filtration chromatograms of the FAW heated at 150 °C for 7 d.

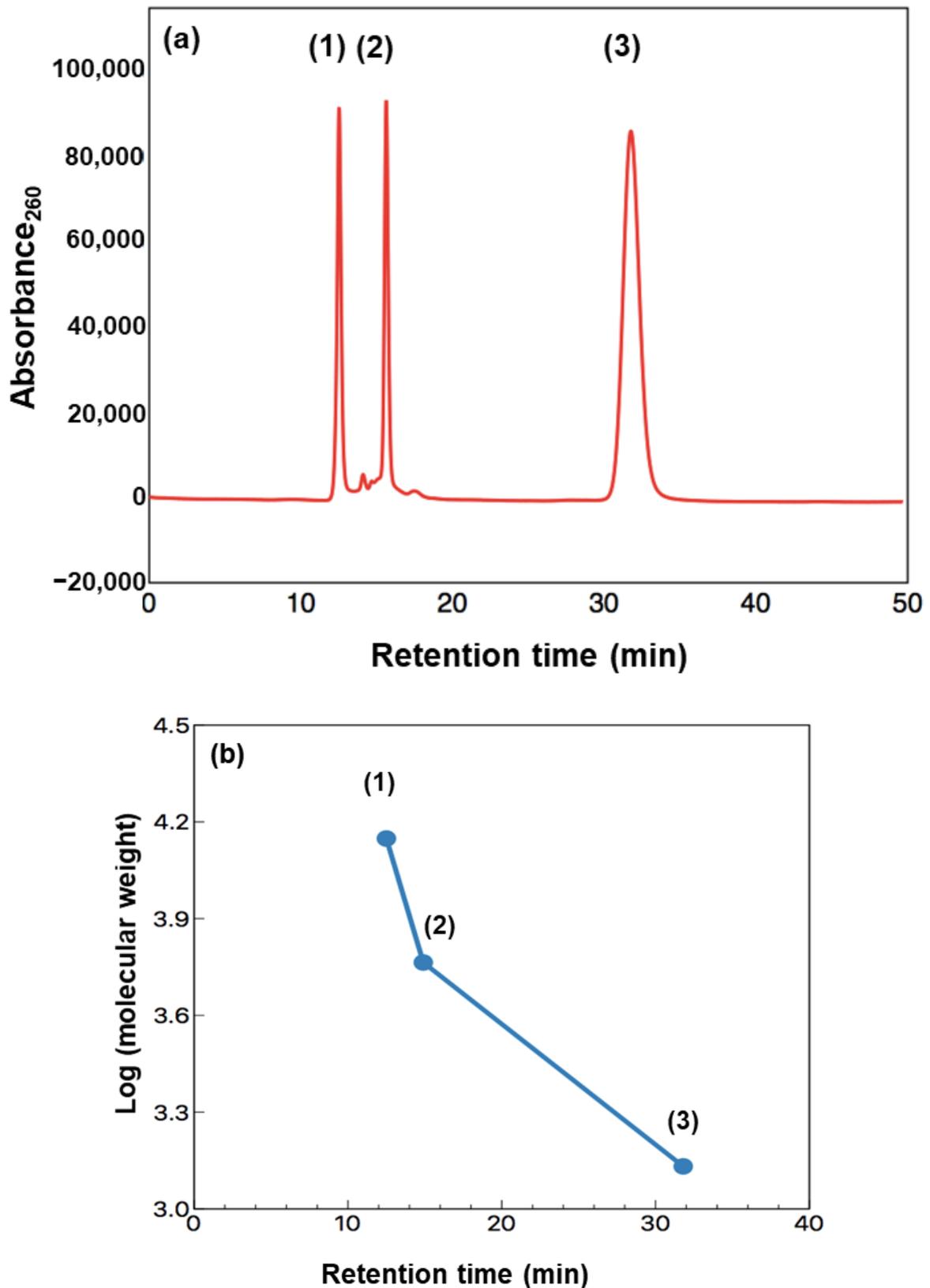


Fig. 3-16 (a) Chromatogram of the protein standard: (1) Lactalbumin (Mwt: 14,073 Da, RT: 12.5 min), (2) insulin (Mwt: 5807 Da, RT: 14.9 min), and (3) vitamin B12 (Mwt: 1355 Da, RT: 31.8 min). (b) Logarithm of the molecular weight of the protein standard as a function of retention time. Note; Mwt: molecular weight, RT: retention time.

The ratios (%) of each peak area among the total areas of the three major peaks were calculated (Fig. 3-17), (Peak 1: 12.6–15.2 min, Peak 2: 15.4–17.5 min, and Peak 3: 17.7–20.1 min). After heating for 1 d, Peak 2 was dominant compared to Peaks 1 and 3 in FAW with and without minerals. After heating from 1 d to 3 d, the area of Peak 1 (highest molecular weight) increased in FAW both with and without mineral addition. However, the area of Peak 3 (lowest molecular weight) increased with mineral addition, while it decreased without minerals. After heating for 7 d, FAW without minerals showed a decrease in Peak 1 (but still higher than that of 1 d heating), in parallel with an increase of Peak 3. On the contrary, adding minerals showed an increase in the area of Peak 1 with a decrease in the area of Peak 3. These results indicate that increasing heating duration without minerals can enhance the combination of organic molecules. However, the minerals enhanced both the decomposition and combination of organic molecules after 3 d, in addition to their combination after 7 d. Overall, the molecular weights of organic molecules could be affected due to the effect of increasing the heating duration as well as the presence of minerals.

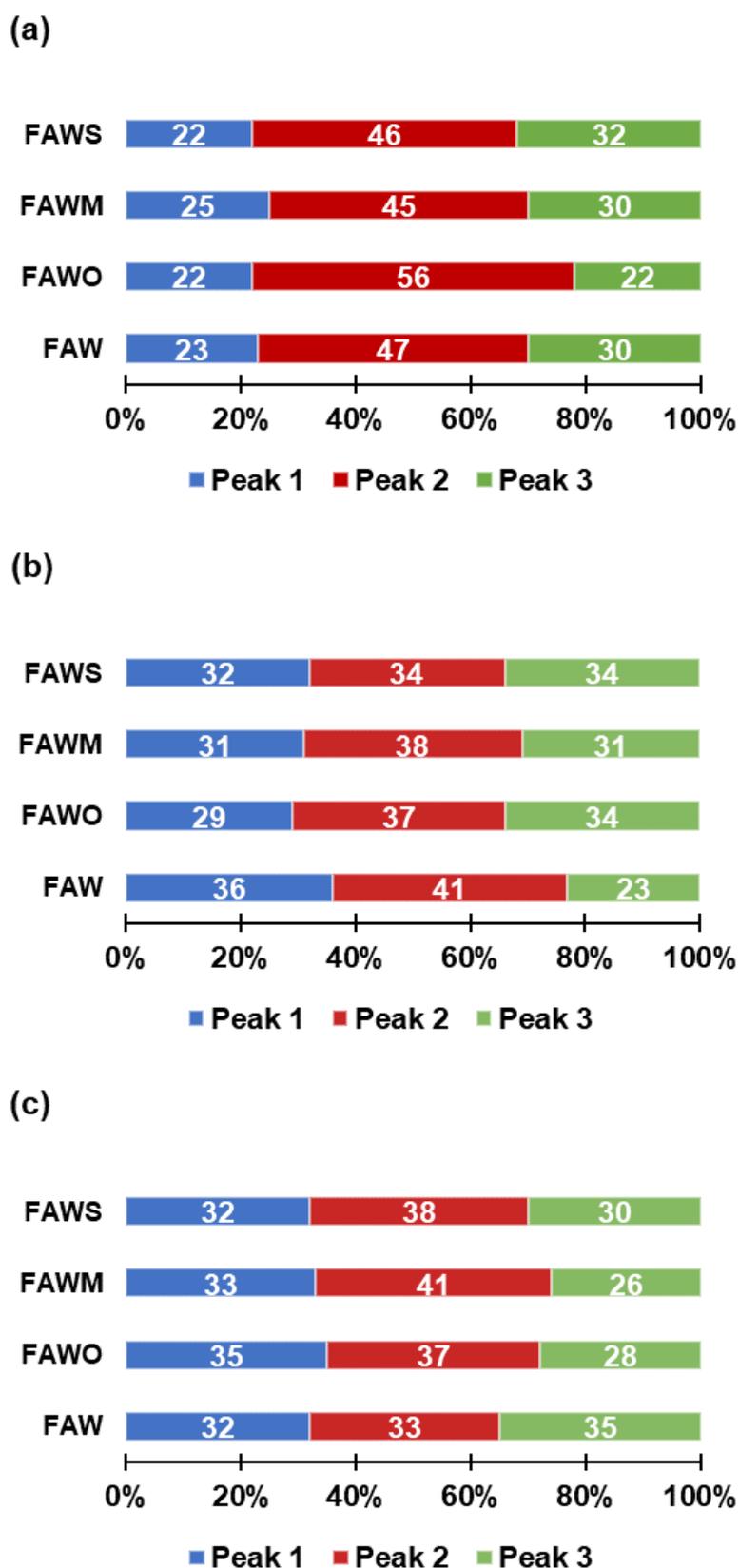


Fig. 3-17 The ratios (%) of each peak area among the total areas of the three major peaks (Peak 1: 12.6-15.2 min, Peak 2: 15.4-17.5 min, and Peak 3: 17.7-20.1 min) of the FAW heated at 150 °C for (a) 1 d, (b) 3 d, and (c) 7 d.

3.4. Discussion

3.4.1. Amino acid production during aqueous alteration in small bodies in the Solar System

Initially, the aqueously altered chondritic parent bodies are composed of silicate-containing icy dust, with the ice consisting of predominantly H₂O, CO, and CO₂ with some formaldehyde and ammonia (Charnley and Rodgers 2008). The internal heating that melted the ice to produce water could be mainly due to the decay of ²⁶Al (Urey 1955; Grimm and McSween 1993), with other possibilities such as impact heating (Rubin 1995). The aqueous alteration conditions have been determined to be mildly alkaline and for CI1 chondrites from 20 °C to 150 °C, for CR chondrites from 50 °C to 150 °C, for CO and CV chondrites from 0 °C to 340 °C, and up to 260 °C for ordinary chondrites (Brearley 2006). Such conditions are favorable for the formose reaction (Kopetzki and Antonietti 2011). Complex macromolecular organic solids were formed by further condensation and carbonization of formaldehyde and glycolaldehyde (Cody et al., 2011). Ammonia could enhance the yields of organic solids and amino acid production (Kebukawa et al., 2013; 2017; Kebukawa and Cody 2015). Therefore, in the present study, I used a mixture of formaldehyde and ammonia in the presence of water for simulating asteroids, the remnants of planetesimals. Moreover, I added some minerals to the previous aqueous mixture to study their catalyzing or inhibiting effects on amino acid production and to analyze in-depth the impact of each mineral. The major amino acid products from my hydrothermal experiments after acid hydrolysis were Gly, Ala, β-Ala, Ser, Asp, Glu, and γ-ABA.

The amino acid production from the hydrothermal experiments conducted for 3 d is approximately ten times less than those in the experimental results of Kebukawa et al. (2017) when they conducted hydrothermal experiments using formaldehyde, glycolaldehyde, ammonia, and water as a starting solution with a molar ratio of C:N:H₂O (7.2:0.72:100) in the presence of saturated Ca(OH)₂. Their results showed that the concentrations of Gly, Ala, and β-Ala heated for 3 d at 150 °C were approximately 300 μM, 500 μM, and 180 μM, respectively. Considering that the C:N:H₂O ratios are close to those in the present experiments (9:1:100), the presence of glycolaldehyde and Ca(OH)₂ could enhance the yields of amino acids. However, the high abundance of glycolaldehyde and Ca(OH)₂ in the meteorite parent bodies are not expected, although glycolaldehyde has been found in comets (Biver et al., 2015). Thus, my starting materials are more realistic for the meteorite parent body conditions. Vinogradoff et al. (2020b) conducted hydrothermal experiments with a starting solution containing hexamethylenetetramine (HMT), and their results showed that, without acid hydrolysis, the

concentrations of Gly, Ala, and β -Ala heated for 2 d at 150 °C were 7.7 μ M, <0.1 μ M, and 1.0 μ M, respectively. Their results are consistent with my 1 d experiment results without minerals since Gly was the dominant amino acid in both studies. However, after increasing the experimental duration to 7 d, their results (Vinogradoff 2020b) showed that Gly increased significantly to 190.6 μ M, still, the dominant amino acid, while Ala and β -Ala increased relatively to become 7.0 μ M and 7.9 μ M, respectively, which is inconsistent with my results in the 7 d experiments, since Ala and β -Ala in my experiments increased notably and became dominants.

In the FAW without minerals, the concentration of Gly revealed some stable behavior over time; however, the concentrations of Ala and β -Ala continuously increased with time. The initial formation of the amino acids could be primarily affected by the heating duration, and thus at 1 d, Gly was the dominant amino acid, yet after 3 d and 7 d, Ala and β -Ala showed a significant increase and became dominant. This could be explained primarily by the amino acid decomposition process during thermal alteration of the organic matter via α -decarboxylation, e.g., the decomposition of Asp leading to the formation of β -Ala (Botta et al., 2002). Many studies have revealed that α -amino acids are abundant in the Murchison meteorite (Botta et al., 2002; 2007; Glavin et al., 2006; 2010), as well as through hydrothermal synthesis experiments formed from a gas mixture of methane and nitrogen with the principal metal ions present in seawater (Yanagawa and Kobayashi 1992), and a solution containing carbonate, cyanide and formaldehyde (Marshall 1994) and a gas mixture of carbon monoxide, ammonia, and water (Elmasry et al., 2020). In contrast, in the CI1 chondrites Orgueil and Ivuna, which were subjected to an extended aqueous alteration compared to CM and CR chondrites, β -Ala is the most abundant (Botta et al., 2002; 2007; Glavin et al., 2006; 2010). This is consistent with the relative decrease of Asp with time in my experiments since β -Ala is formed by the decomposition of Asp.

3.4.2. The effects of minerals on amino acid formation

My results from the hydrothermal experiments with the presence of minerals showed that olivine, montmorillonite, and serpentine enhance the amino acid yield at a short heating duration (1 d). Adding montmorillonite to the FAW solution mixture could enhance amino acid production remarkably more than olivine and serpentine after heating for 1 d. My results are supported by previous studies that demonstrated that montmorillonite enhanced the yield of amino acids in prebiotic conditions simulating early Earth (Shimoyama et al., 1978; Hashizume

2012). Amino acid preservation is probably promoted by montmorillonite due to its high surface areas and small pore sizes (Dos Santos et al., 2016).

However, after 3 d and 7 d, the formation of the amino acids decreased significantly with montmorillonite; thus, the decomposition of amino acids could be enhanced by montmorillonite at longer heating durations. Moreover, the production of amino acids was enhanced in the presence of olivine and serpentine at 1 d and 3 d, yet with a longer heating period (7 d), the presence of these minerals decreased amino acid production enhancement. Despite this, while in the presence of olivine, the amino acid concentration decreased after 3 d, it was still higher than for FAW without minerals, and consequently, olivine and serpentine were considered to enhance amino acid production at 1 d and 3 d. After 7 d, all minerals had a negative effect on amino acid production, as they enhanced amino acid decomposition. Overall, it was suggested from my results that minerals enhanced the production of amino acids at short heating durations yet enhanced their decomposition for longer heating durations. It should be noted that my experiments were conducted at relatively high temperature (150 °C) with much shorter duration (days) to enhance the reaction more quickly compared to the parent body process, which proceeded at a lower temperature, possibly close to 0 °C up to 150 °C, with much longer duration, could be millions of years. Such environments cannot be simulated in laboratory experiments, and thus to discuss more realistic and detailed time-temperature dependence of amino acid behavior, kinetics, and thermodynamic evaluations would be required.

Meteoritic organic matter is closely correlated with phyllosilicates, proposing that these minerals may have had catalytic effects during molecular evolution in the early solar system (Pearson et al., 2002). It has been proposed that phyllosilicates have catalytic effects on the synthesis and polymerization of amino acids in the prebiotic Earth environment, e.g., (Ponnamperuma et al., 1982; Lambert 2008; Marshall-Bowman et al., 2010). The surfaces of phyllosilicates are elementally charged in an aqueous solution, and then amino acids are adsorbed on their surfaces. The adsorption mechanisms of amino acids and phyllosilicates have also been studied (Lambert 2008; Stievano et al., 2007; Meng et al., 2004; Parbhakar et al., 2007; Kitadai et al., 2009). The chemical characteristics of specific mineral adsorption sites may affect the determination of amino acid stability. Vinogradoff et al. (2020a; 2020b) conducted hydrothermal experiments at 150 °C and an alkaline pH, using HMT, in the presence of Al- and Fe-rich phyllosilicates (smectites), finding the decomposition of HMT to formaldehyde and ammonia, leading to the formation of a diverse suite of soluble organic

compounds, including amino acids, which were more abundant and more complex, in the presence of smectite. They found that Fe-rich smectite also prohibited the production of amino acids, while the presence of Al-rich smectite stimulated it after 31 days (Vinogradoff et al., 2020b). Thus, the production of the amino acids could be affected by the unique characteristics of smectites, such as structure, chemical composition, and crystal size. In my hydrothermal experiments at 150 °C and an alkaline pH, using formaldehyde and ammonia, the presence of smectite (montmorillonite) induced the enhancement of amino acid production at 1 d and the decomposition of amino acids after longer heating. This could be due to the changes in the structure of montmorillonite during heating.

3.4.3. Molecular structure variations of FAW with minerals

Here I discuss the bulk molecular structures of FAW, which include the amino acid precursors based on the IR spectra of the dried solutions. The ester C=O/C=C peak intensity ratios in FAW without minerals showed a higher value after heating for 1 d and 3 d than these ratios in FAW with minerals. This could be due to the enhancement of the decarboxylation by the minerals at short heating durations; while, after heating for 7 d, the addition of minerals seems to increase this ratio, which may be due to catalytic esterification at longer heating durations. The presence of both Brønsted and Lewis acid sites in phyllosilicates can make them natural esterification catalysts (Varma 2002). The sources of Brønsted and Lewis acidity are the hydrated and dehydrated cations, respectively, in the interlayer space and the surface of phyllosilicates (Wu et al., 2012). Olivine also contains Lewis acid sites (Tian et al., 2012), which promote the esterification of FAW.

The peak intensity ratio of CH₂/CH₃ is a good index of the length and branching of the aliphatic chain. The CH₂/CH₃ peak intensity ratio is not equal to its molar abundance ratio due to the difference in molar absorption coefficients between CH₂ and CH₃; however, there is a linear correlation between the CH₂/CH₃ peak intensity ratio and the actual number of CH₂/CH₃ in molecules (Igisu et al., 2009). Higher CH₂/CH₃ ratios refer to longer aliphatic chains or higher contents of cyclic aliphatic structures, whereas shorter chain lengths or higher branching levels are suggested by lower CH₂/CH₃ ratios. The average CH₂/CH₃ peak intensity ratios of the FAW with minerals heated for 1 d, 3 d, and 7 d were 1.445 ± 0.005 , 1.291 ± 0.003 , and 1.251 ± 0.006 , respectively. The CH₂/CH₃ ratio in heated FAW with minerals was higher than that ratio without minerals for at least the 1 d and 3 d heating durations (Fig. 3-12d), suggesting that the minerals enhance the aliphatic chain length.

Interestingly, The CH₂/CH₃ peak intensity ratios of the FAW were consistent with the reported CH₂/CH₃ peak intensity ratio of the IOM from the least heated chondrites (1.2–1.4) (Kebukawa et al., 2011) within analytical error, although my data was from soluble fractions and a large fraction of organic matter in meteorites are insoluble. Therefore, this implies that there is a link between the soluble organic compounds in FAW and that of the IOM, and perhaps the soluble organics condensed into large macromolecules and became IOM-like and/or perhaps some fraction of soluble organics was produced as an alteration product of IOM by parent body processes (Sephton et al., 2003; Huang et al., 2007).

Adding minerals could affect the nature of C=C since it was suggested that a higher wavenumber indicated a more olefinic nature, and a lower wavenumber indicated a more aromatic nature (Socrates 2004). The wavenumber of the C=C peaks decreased after adding minerals at 1 d (Table 3-3), and therefore C=C in FAW without minerals has a more olefinic nature. This then became more aromatic after adding minerals, which might be due to cycloaddition enhancement by the minerals. Phyllosilicates are recognized as effective for redox reactions, including cycloadditions (McCabe 1996), due to the existence of both Brønsted and Lewis acid sites (Houk and Strozier 1973). Besides, increasing the heating duration could also affect the nature of C=C in FAW without minerals and FAW with olivine, since their wavenumber decreased and became more aromatic at longer heating durations; however, FAW with phyllosilicates did not show any difference with heating. The C=O (ester) shifted slightly to a higher wavenumber (1760 cm⁻¹ to 1770 cm⁻¹) due to the presence of minerals as well as increased heating duration. This slight increase in the wavenumber of the peaks in FAW with minerals and after longer heating refers to an increase in the force constant of that bond, which suggests the enhancement of bond strength (Trivedi et al., 2015); the increase in the force constant of bonding peaks is likely to refer to an increase in the rigidity of bonds in the molecule (Guan et al., 2007).

High molecular weight organic compounds, estimated at several hundred or a few thousands, were formed in these hydrothermal experiments, which indicated that a solution mixture of formaldehyde and ammonia in the presence of water might be characterized as amino acid precursors that remain stable at longer heating durations, providing various amino acids after acid hydrolysis. The minerals used in this study, olivine and phyllosilicates (montmorillonite and serpentine), might have mostly similar effects and seem to enhance both the decomposition and combination of organic molecules.

3.5. Conclusions

Hydrothermal experiments were conducted using a mixture of formaldehyde and ammonia in the presence of water to simulate aqueous alteration in small bodies in the Solar System. Since the presence of minerals may play significant roles in the formation of organics as well as their evolution in the early Solar System through the catalyzation of various organic reactions, three kinds of minerals were added one by one to the mixture solution of H_2CO , NH_3 , and water to evaluate their enhancing and inhibiting effects on amino acid production. The FAW produced various kinds of amino acids after acid hydrolysis. Amino acids released after the heating experiments showed different behaviors: Gly remained mostly stable over time while Ala and β -Ala showed a significant increase after 7 d of heating. The minerals enhanced the formation of amino acids at shorter heating durations (1 d), especially with montmorillonite, where the amino acid production was the highest among all tested samples; however, after 3 d and 7 d, the formation of the amino acids decreased significantly with montmorillonite. Olivine and serpentine enhanced amino acid production for 1 d and 3 d heating durations. After 7 d, all minerals had a negative effect on amino acid production, as they enhanced amino acid decomposition. The total concentration of amino acids with olivine and serpentine showed stable behavior with time at around 40 μM for 1 d to 7 d. Accordingly, I suggest that minerals enhance the amino acid production at a short heating duration and enhance their decomposition for longer heating durations. I proposed that the concentrations of the amino acids depend on (1) the presence and the kind of minerals and (2) the stability of the amino acid during longer heating durations. IR spectra of the soluble fraction of FAW (dried) revealed that the ester $\text{C}=\text{O}/\text{C}=\text{C}$ peak intensity ratios in the presence of minerals were lower at short heating duration, which might be due to the enhancement of decarboxylation by minerals, and increased after heating for 7 d, which might be due to catalytic esterification by minerals. Gel filtration chromatography indicated that high molecular weight organic compounds were formed from the FAW solution mixture, which started to decompose after a longer heating duration (7 d) without minerals, while the addition of minerals enhanced both the decomposition and combination of organic molecules. Finally, my data showed that minerals affected the formation of amino acids in aqueous environments simulating small bodies in the early Solar System, and the amino acids could have different response behaviors according to the presence of various minerals.

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Chapter 4

Thermodynamic model and its experimental correlation for the effect of minerals on amino acid formations in hydrothermal environments simulating terrestrial and extraterrestrial water worlds

Elmasry W, Kebukawa Y, Shibuya T, Makabe A, Kobayashi K. Thermodynamic model and its experimental correlation for the effect of minerals on amino acid formations in hydrothermal environments simulating terrestrial and extraterrestrial water worlds. "In preparation"

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Abstract

The delivery of the organic compounds, including amino acids, in meteorites to the early Earth, may have played an important role in the origin of life. Therefore, there is a continuous interaction between water and minerals, which leads to a gradual aqueous alteration to different degrees in carbonaceous chondrite. Subsequently, continuous interaction between organic matter and minerals indicates that minerals have an effect on the formation of amino acids in aqueous environments in small Solar System bodies and that the amino acids could have different response behaviors according to various minerals. In this study, I investigated the catalyzing/inhibitory impact of minerals on amino acid production thermodynamically by using Geochemist's Workbench 15.0 (GWB 15.0) software supported by laboratory experiments. Various amino acids have been synthesized from environments simulating aqueous alteration in small bodies by using a solution mixture of H_2CO and NH_3 in presence of water. In both the presence and absence of minerals, glycine was the most abundant amino acid, but its abundance was very low compared to those produced by laboratory experiments. Minerals enhanced the yield of glycine at a short heating duration (1 d); however, they induced its decomposition at a longer heating duration (7 d). Furthermore, by raising the temperature (25 °C–300 °C), olivine enhanced the production of amino acids, while phyllosilicates enhanced their decomposition at a temperature of more than 150 °C. According to my results, the formation of amino acids in aqueous environments in small Solar System bodies was affected by starting materials concentrations and molar ratios, CO_2 and CO concentration, Formate and acetate concentrations, temperature, heating duration, and different minerals.

4.1. Introduction

Various organic molecules have been discovered in interstellar space, which they were abiotically created in the early Solar System, then accreted into parent bodies of meteorites and processed, and eventually delivered to the early Earth. Formaldehyde and ammonia are widely distributed in the interstellar medium and some primitive Solar System bodies such as comets. Recently, it is suggested that some of the meteoritic organic matter was produced through reactions of formaldehyde (Cody et al., 2011). Furthermore, Kebukawa et al. (2013, 2017) illustrated that the presence of ammonia enhances the yields of organic matter from formaldehyde, and simultaneously producing amino acids.

During the hydrothermal processes, minerals in parent bodies could play an important role in the evolution of meteoritic organic matter. The aqueously altered meteorites contain up to

1.3 wt.% H in water/OH mainly in the structure of phyllosilicates (Alexander et al. 2013). Meteoritic organic matter is closely correlated with phyllosilicates, proposing that these minerals may have had catalytic effects during molecular evolution in the early solar system (Pearson et al. 2002; Vinogradoff et al. 2020a; 2020b). Thus, the production of the amino acids could be affected by the unique characteristics of phyllosilicates, such as structure, chemical composition, and crystal size. Yamashita and Naraoka (2014) demonstrated that the presence of olivine powder promoted the formation of alkylpyridines from aldehydes and ammonia (the Chichibabin synthesis).

There are considerable studies focused on the thermodynamic calculation of the chemical interactions between rock-forming minerals and aqueous solutions (e.g., Zolotov et al. 2006; Sekine et al. 2015; Kitadai et al. 2021). From thermodynamic analysis of secondary mineralization in CV chondrites, Zolotov et al. (2006) used thermodynamic modelling to demonstrate that aqueous alteration of CV chondrites occurred below ~ 350 °C, at a total pressure below 100 bars and with a relatively low water/rock ratio (<0.2). The thermodynamic calculations using the Geochemist workbench (GWB) were used to model aqueous alteration in different environments. For example, Rosenberg et al. (2001) used it to simulate aqueous processes on CM chondrites, and their results support a scenario in which the major alteration minerals found in CM carbonaceous chondrites were produced by low-temperature (0-25°C) aqueous alteration of an anhydrous CM asteroidal parent body and water. Ohnishi and Tomeoka (2010) used GWB to investigate the alteration products of enstatite formed under various pH, temperature, and run-duration conditions, to compare the alteration products with those in the aqueously altered carbonaceous chondrites. Their results (Ohnishi and Tomeoka 2010) indicate that alteration of enstatite is strongly dependent on the experimental conditions, especially pH. Besides, the ocean chemistry of Enceladus was designed using a thermodynamic model (GWB), and the results showed the presence of silica particles which are considered as evidence of the presence of hydrothermal activity (Sekine et al. 2015).

In my previous hydrothermal experiments (more details in chapter 3) at 150 °C and an alkaline pH, using formaldehyde and ammonia, the presence of montmorillonite induced the enhancement of amino acid production at 1 d and the decomposition of amino acids after longer heating (Elmasry et al. 2021). This could be due to the changes in the structure of montmorillonite during heating. By using phyllosilicates and olivine, I implied that minerals showed both positive and negative effects on the amino acid formation (Elmasry et al. 2021) under the limited experimental conditions. Overall, the presence and the nature of the silicates could affect the abundance and molecular composition of the final organic compounds in

primitive bodies during hydrothermal alteration. Despite ongoing research, the effects of minerals on amino acids production are still not well known and need further studies.

The numerical calculation provides us valuable information that difficult to be obtained from the experimental analysis only due to the limitation of the analytical laboratory devices, hence I could change the reaction conditions easily and obtain the optimum conditions that should be considered for the formations of the amino acids. In addition, It provides additional information about the availability, redox behavior, and fate of chemical species (in particular, amino acids) for longer reaction durations (i.e., parent body processes lasted millions of years), and the physicochemical properties of hydrothermal systems (temperature and pH). In this study, the numerical calculation was conducted to study the impact of the minerals on the production of amino acids in the early solar system.

4.2. Method

4.2.1. Thermodynamic modelling software

I conducted a laboratory-based simulation experiment using the commercial software Geochemist's Workbench 14.0 (GWB 15) and the experimental procedures are summarized in Figure 4-1. This software uses the thermodynamic database (thermo. Com.V8.R6.full), which allows the computation of activity coefficients with a Debye–Huckel model. Species that are known to not form in the aqueous environment could be suppressed to avoid confusion. The results were obtained by using the SUPCRT92 code (Johnson et al., 1992).

GWB is a set of software tools for handling chemical reactions, calculating stability diagrams and the equilibrium states of natural waters, tracing reaction processes, modeling reactive transport, plotting the results of these calculations, and storing the related data (Bethke 2021). It is used to create environmental models producing graphical and textual outputs. It has several subprograms, e.g., ACT2, REACT, TACT, RXN, and GTPLOT. In my research, I used the subprogram REACT, which is used for reaction modelling. REACT calculates species distributions in aqueous solutions and computes mineral saturation and gas fugacity and traces the reaction paths involving fluids, minerals, and gases. Then, the results of the simulation can be made graphically by using an interactive program called GTPLOT.

The starting materials are composed of formaldehyde, ammonia, and water simulating planetesimals, in a molar ratio of 9 : 1 : 100 under alkaline conditions (pH 10). The starting materials were treated with thermal cycling (25 °C to 300 °C), with and without adding different kinds of minerals (10 g/L) solution.

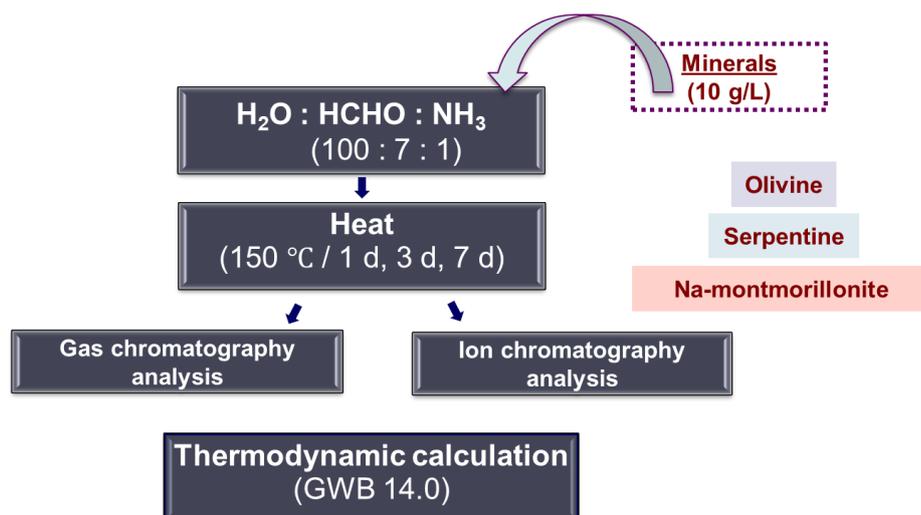


Fig. 4-1 Summary of experimental flows.

4.2.2. Laboratory experiments

To constrain the hydrothermal reaction conditions on parent bodies of meteorites and make the reaction model calculations more real and comparable with the experimental results, I conducted gas chromatography (GC) analysis and ion chromatography (IC) analysis to analyze the gas concentrations and cation and anion concentrations, respectively, in the hydrothermal fluid.

The sealed tubes should be opened in a gas extraction system to avoid outside atmospheric air mixing with the gases within the sealed tubes and/or gases leakage during the opening process. The gas extraction system (Fig. 4-2) is composed of an evacuator, which is a vacuum pump with attached gas-tight valves. Also, there is a vacuum gauge, which monitors the pressure to which I evacuate the vials. After the evacuation, the sealed tubes were cracked, and the liberated gas was trapped in a stainless-steel tube immersed in liquid helium. Wait for 40 min at room temperature for homogenization of the gas mixture. The liberated gas was directly collected from the sealed tubes containing a heated solution mixture of formaldehyde and ammonia in presence of water (FAW) by using a gas-tight syringe.

For pH analysis, a sample solution (500 μ L) was analyzed by a pH meter, which range measurement was from 2 to 12, and the resolution was 0.1 unit.



Fig. 4-2 Gas Extraction System in JAMSTEC

4.2.3. Gas Chromatography (GC) analysis

Concentrations of dissolved gas species in the hydrothermal fluid were monitored by using a gas chromatograph Nexis (GC-2030 Shimadzu) at JAMSTEC, Japan Agency for Marine-Earth Science and Technology. This system is equipped with a TCD (thermal conductivity detector) and helium as a carrier gas. A 100 μl gas sample was collected, then injected directly to GC. Gas species were identified by retention time comparison with known gas mixture standard.

4.2.4. Ion Chromatography (IC) analysis

Anion concentrations in the hydrothermal fluid were measured by using ion chromatography (DIONEX ICS-2100) system at JAMSTEC, Japan Agency for Marine-Earth Science and Technology. This system is equipped with an IonPac AS11-HC anion exchange column with an Eluent Generator which is used to produce KOH gradients from deionized water. An electric conductivity detector and an auto-sampler (DIONEX AS-AP) were used to

analyze FAW samples which were diluted with Milli-Q water, filtered before the analysis, and the sample injection volume was 10 μ l. Formate and acetate were calibrated against standard mixture from Wako Chemicals, Japan.

4.2.5. Thermodynamic calculation

The data that was collected from laboratory experimental results, was inserted into the GWB input file for the program REACT (Table. 4-1) with starting materials: formaldehyde, ammonia, and water, in a molar ratio of 9 : 1 : 100 under alkaline conditions (pH 10). Starting materials treated with thermal cycling (RT to 300 °C), with and without adding different kinds of minerals (10 g/L) solution.

Table. 4-1 GWB input data with starting materials: formaldehyde, ammonia, and water

Starting solution (mol/L)	HCHO (3.89) / NH ₃ aq (0.56) in presence of water
Log f O ₂ (g)	1.0e ⁻⁴⁰
Temp (°C)	25—300
pH	10
Balancing species: Na ⁺ , Cl ⁻ (mmol/kg)	1.0e ⁻⁴⁰
CO ₂ g (mg/m ³)	224800
CO g (mg/m ³)	2333
Acetate (mmol/L)	96.5
Formate (mmol/ L)	139.6

4.3. Laboratory experimental results

4.3.1. Ion concentrations

For peak identification, standard solutions of anions were used (Fig. 4-3). The detected anions in the standard solution were Lactate, Acetate, Formate, Pyruvate, Cl⁻, and Br⁻. Figures 4-4—4-6 display acetate and formate chromatograms of the FAW with and without minerals heated at 150 °C for 1 d, 3 d, and 7 d, comparing the addition of different minerals. Figure 4-7 showed the concentrations of acetate and formate in the hydrothermal solution of FAW that heated at 150 °C with and without minerals under for 1 d, 3 d, and 7 d.

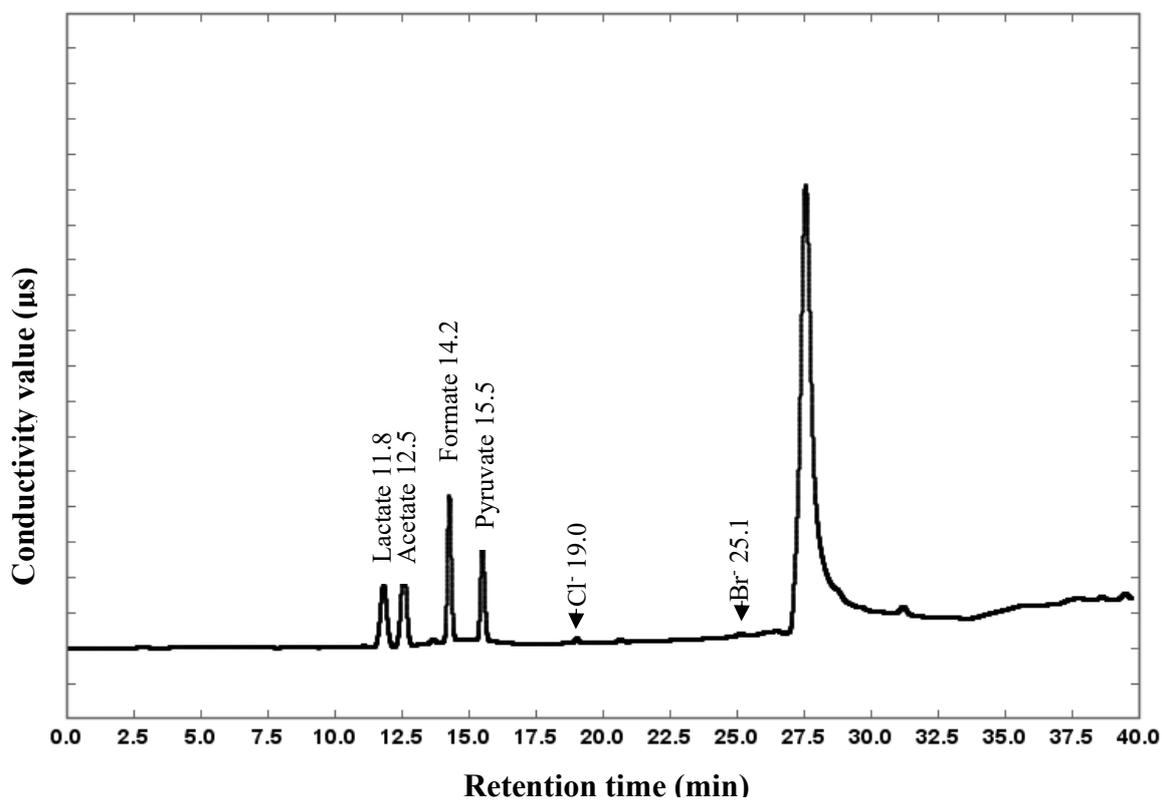


Fig. 4-3 Chromatogram of the anion mixture standard (0.001 mM)

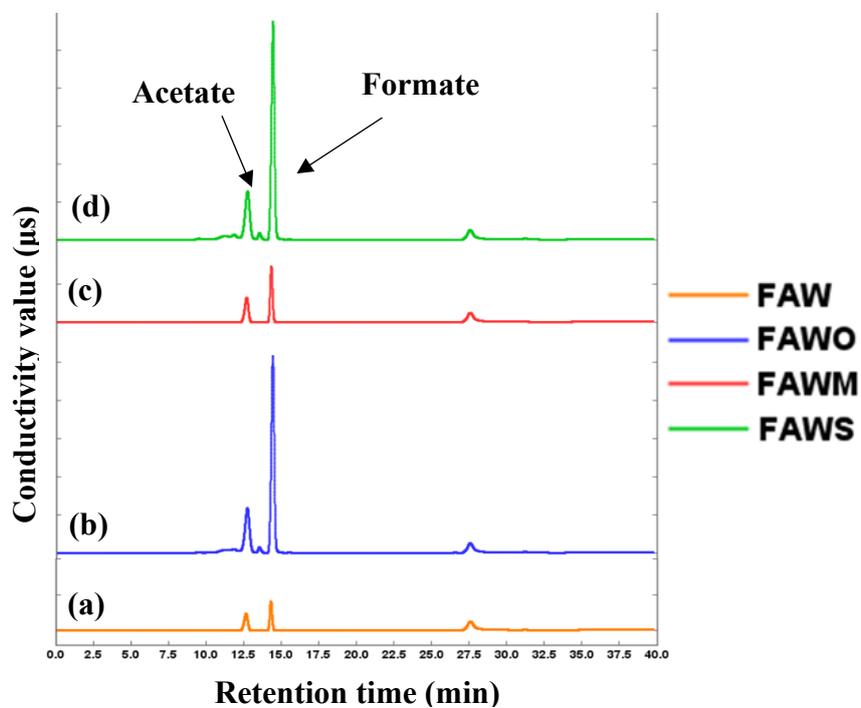


Fig. 4-4 Chromatograms of Acetate and Formate in the heated at 150 °C for 1 d under the following conditions: (a) without minerals “FAW” (b) with adding montmorillonite “FAWM” (c) with adding olivine “FAWO”, and (d) with adding serpentine “FAWS”

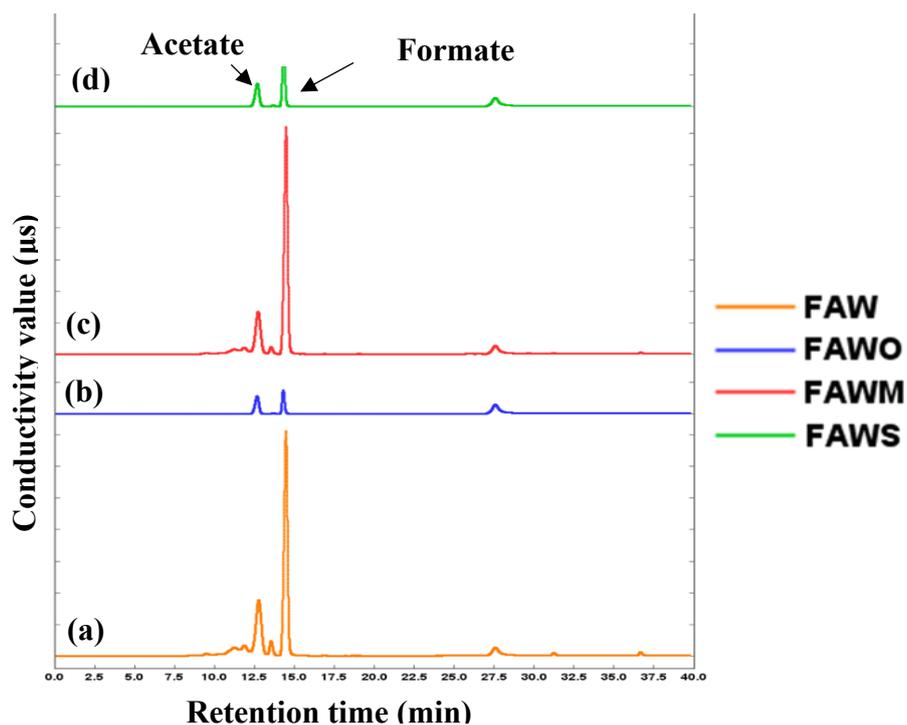


Fig. 4-5 Chromatograms of Acetate and Formate in the heated at 150 °C for 3 d under the following conditions: (a) without minerals “FAW” (b) with adding montmorillonite “FAWM” (c) with adding olivine “FAWO”, and (d) with adding serpentine “FAWS”

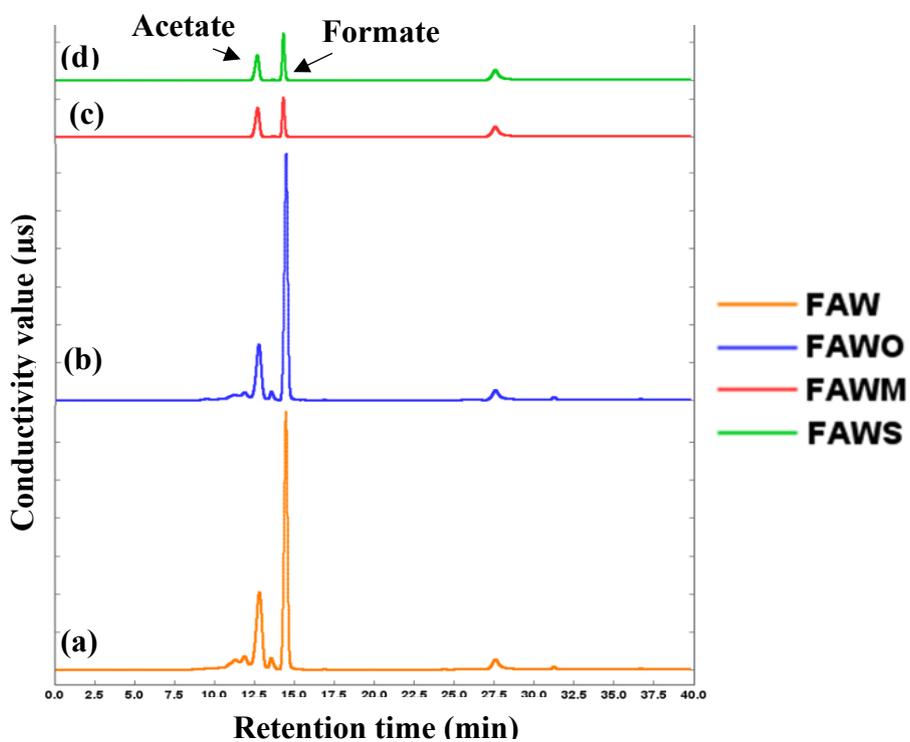


Fig. 4-6 Chromatograms of Acetate and Formate in the heated at 150 °C for 7 d under the following conditions: (a) without minerals “FAW” (b) with adding montmorillonite “FAWM” (c) with adding olivine “FAWO”, and (d) with adding serpentine “FAWS”

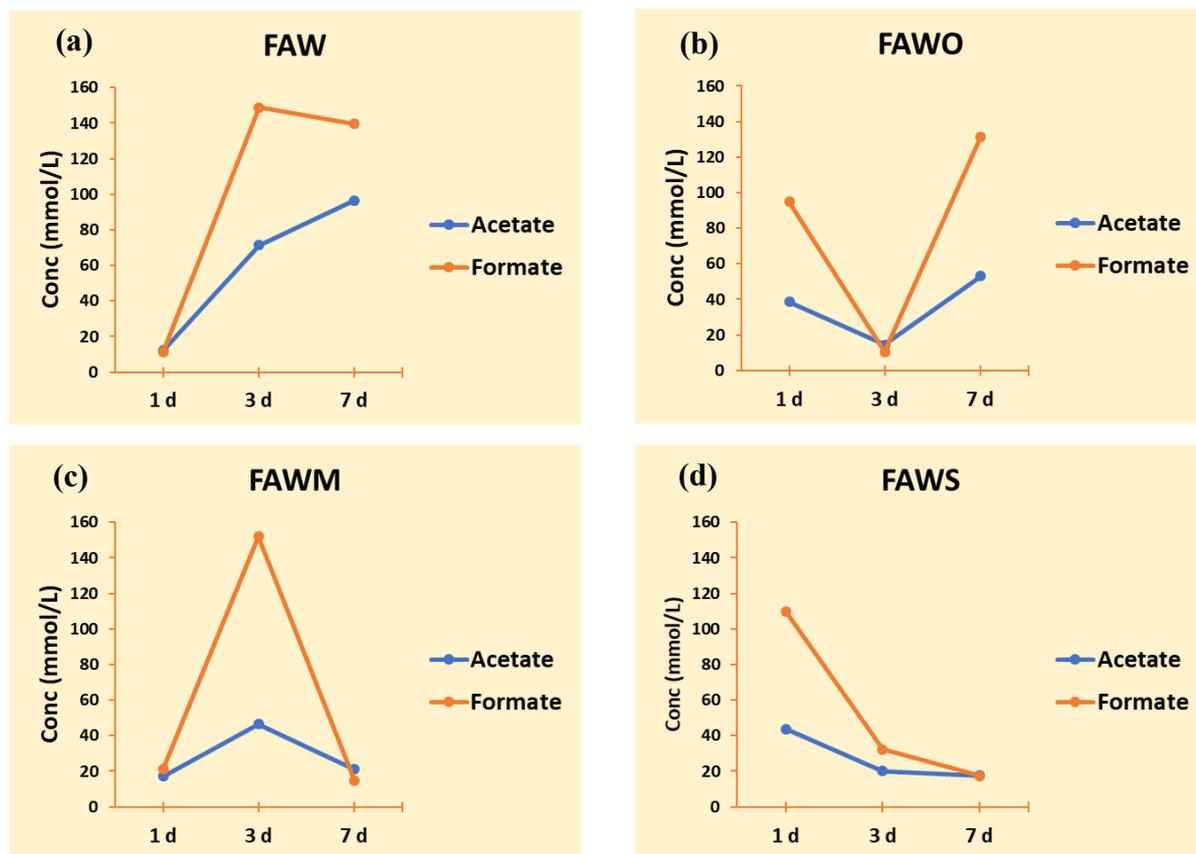


Fig. 4-7 Acetate and formate concentrations (mmol/L) in the hydrothermal solution under the following conditions: **(a)** without minerals “FAW” **(b)** with adding montmorillonite “FAWM” **(c)** with adding olivine “FAWO”, and **(d)** with adding serpentine “FAWS”

4.3.2. Gas concentrations

The main gases in the heated samples are CO₂ and CO with a very low contribution of H₂ and CH₄ (Fig 4-8, Table 4-2).

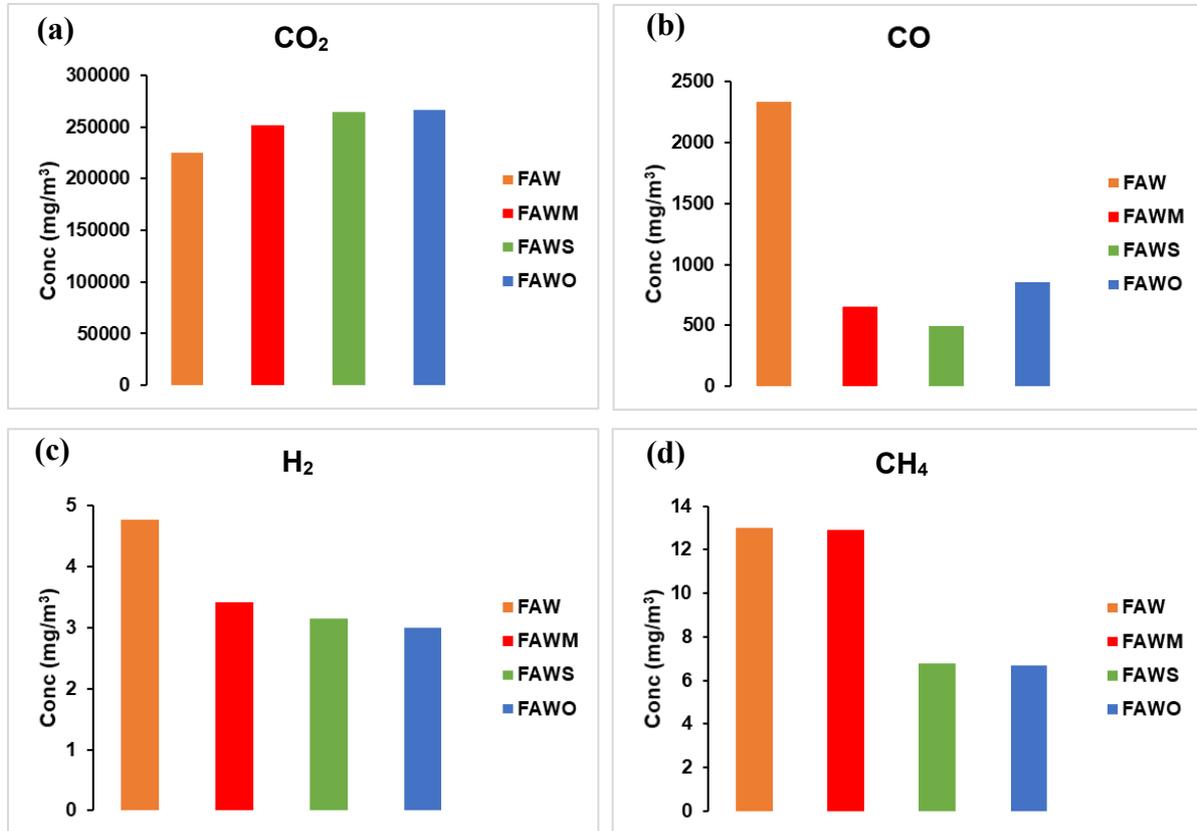


Fig. 4-8 Concentrations (mg/m³) of (a) CO₂, (b) CO, (c) H₂, and (d) CH₄ in FAW samples heated at 150 °C for 7 d (Note: FAW: without minerals, FAWO: with olivine, FAWM: with montmorillonite, FAWS: with serpentine).

Table 4-2 Gas concentrations in FAW samples heated at 150 °C for 7 d

Concentration (mg/m ³)	FAW	FAWO	FAWM	FAWS
CO ₂	224800	266700	252050	264150
CO	2333.0	857.8	657.4	497.6
H ₂	4.8	3.0	3.4	3.2
CH ₄	13.0	6.7	12.9	6.8

4.4. Thermodynamic calculation results

various amino acids have formed from FAW aqueous solution with the predominance of Gly and Ala. All amino acids increased to $\sim 150^{\circ}\text{C}$, whereas decreased with increasing the temperature more than 150°C (Fig. 4-9). The calculated concentrations of amino acids were lower than those obtained through laboratory experiments. It probably due to the hydrolysis step in the laboratory analysis that liberates large amounts of amino acids, while the calculation treated the formation of free amino acids only, not amino acid precursors. The numerical calculation showed roughly the same behavior for my previous experimental analysis (Elmasry et al. 2021), as the FAW without minerals enhanced the Gly production with time (Fig. 4-10), while phyllosilicates enhanced its decomposition after 7 d. Olivine demonstrated some stability in Gly concentration over time. It should be noted that the Gly concentration (Fig. 4-10) at different periods (1 d–7 d) was calculated based on the results of laboratory analysis of carbon species, formate, and acetate concentration in the FAW samples. Since we cannot control the time in the software, therefore, I inserted the different results that were obtained from the laboratory experiments at 1 d, 3 d, and 7 d in the input file of the GWB software. Further simulation experiments needed to be conducted in order to examine the effect of different chemical parameters, e.g., temperature and pH, on the stability of the amino acids as well as the alteration of minerals.

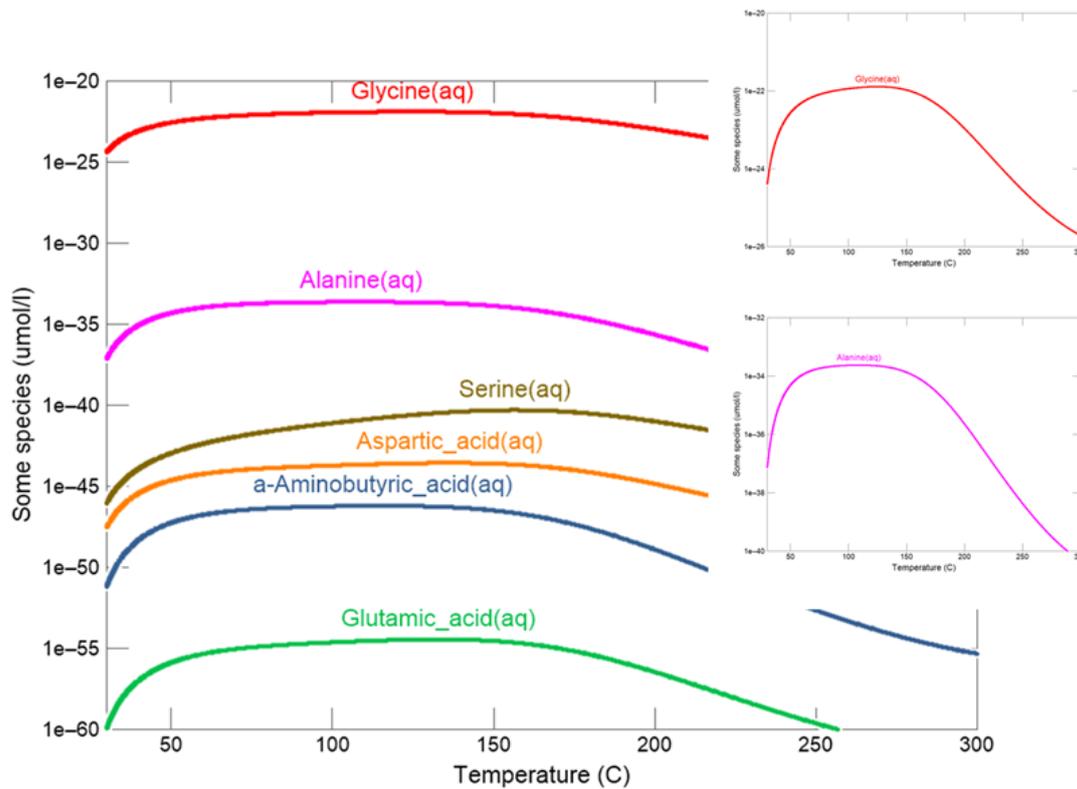


Fig. 4-9 Concentration of amino acids (calculated by GWB) in the FAW system without minerals over thermal cycling (25 °C to 300 °C).

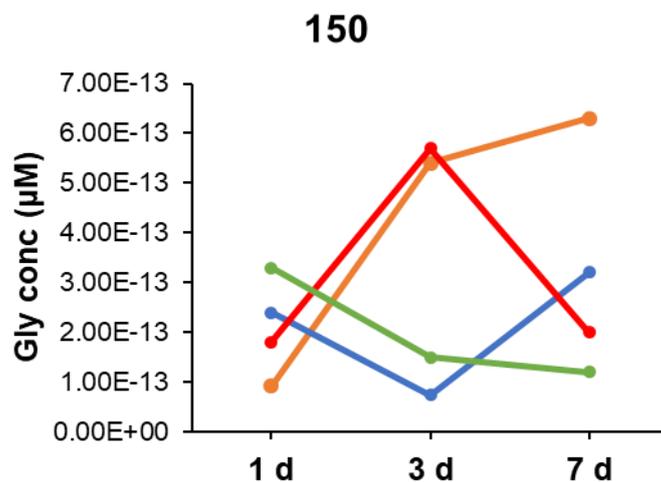


Fig. 4-10 Glycine concentration (calculated by GWB) in the FAW system at 150 °C for 1 d, 3 d, and 7 d. (It should be noted that these results were calculated based on the laboratory analysis of gaseous species, anions, and cations in the FAW samples).

Minerals have different effects on amino acid production with increasing the temperature, since olivine enhanced the yields of amino acids with Gly and Ser as the predominates (Fig. 4-11), while the production of amino acids decreased with heating in presence of phyllosilicates (Figs 4-12,4-13) with Gly and Ala as the predominates.

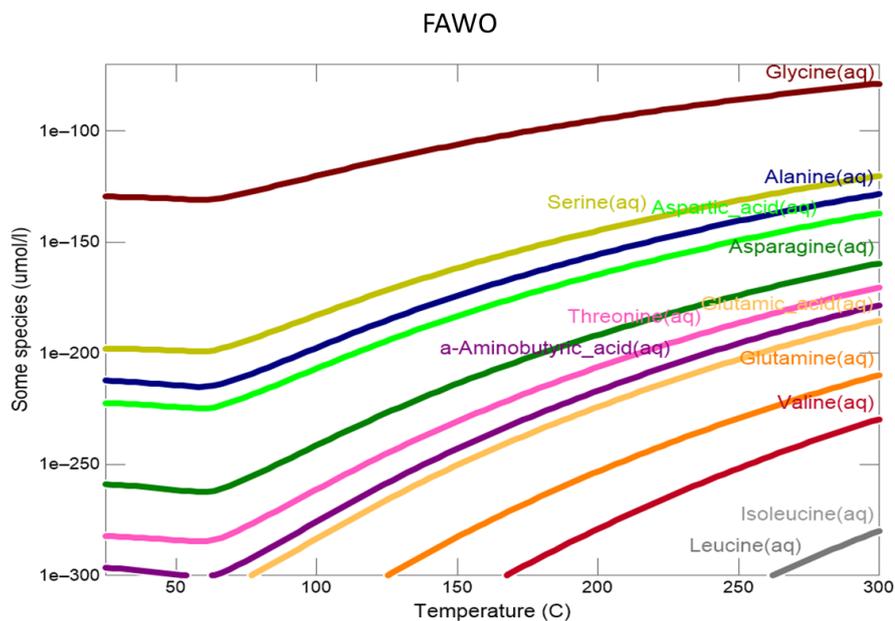


Fig. 4-11 Concentration of amino acids produced in the FAW hydrothermal solution system with olivine over a temperature (25 °C to 300 °C).

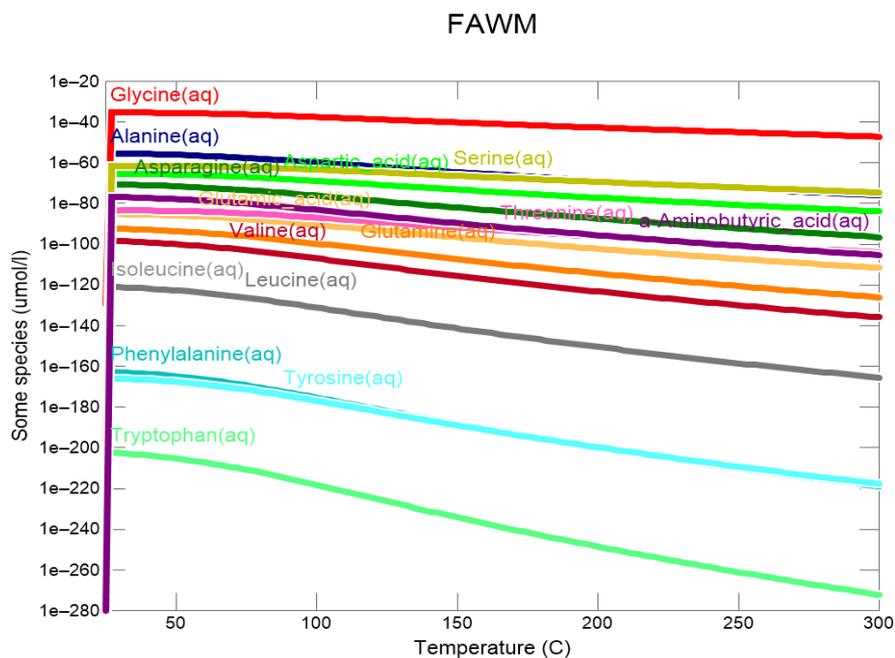


Fig. 4-12 Concentration of amino acids produced in the FAW hydrothermal solution system with montmorillonite over a temperature (30 °C to 300 °C).

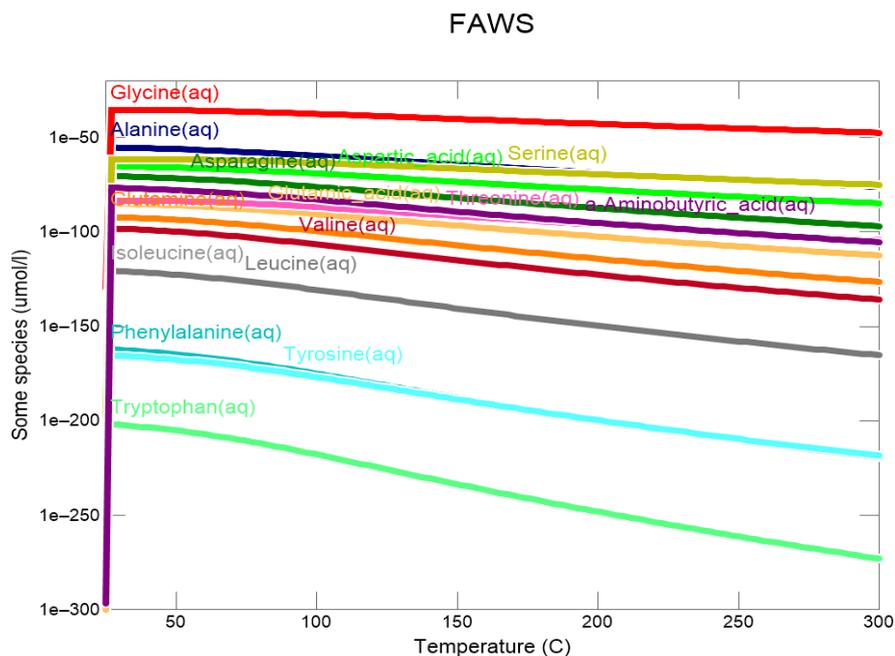


Fig. 4-13 Concentration of amino acids produced in the FAW hydrothermal solution system with serpentine over a temperature (30 °C to 300 °C).

4.5. Discussion

The aqueously altered chondritic parent bodies are composed of silicate-containing icy dust, with the ice consisting of predominantly H₂O, CO, and CO₂ with some formaldehyde and ammonia (Charnley and Rodgers 2008). The aqueous alteration conditions have been determined to be mildly alkaline and for CI1 chondrites from 20 °C to 150 °C, for CR chondrites from 50 °C to 150 °C, for CO and CV chondrites from 0 °C to 340 °C, and up to 260 °C for ordinary chondrites (Brearley 2006). Such conditions are favorable for the formose reaction (Kopetzki and Antonietti 2011). Complex macromolecular organic solids were formed by further condensation and carbonization of formaldehyde and glycolaldehyde (Cody et al., 2011). Ammonia could enhance the yields of organic solids and amino acid production (Kebukawa et al., 2013; 2017; Kebukawa and Cody 2015). Therefore, in the present study, I used a mixture of formaldehyde and ammonia in the presence of water for simulating asteroids, the remnants of planetesimals. Moreover, I added three kinds of minerals to the simulation model to study impacts on amino acid production.

Gly was the dominant amino acid since the abiotic synthesis follows the rules of thermodynamics and results in an abundance the simplest amino acid (Gly) relative to the others (Higgs and Pudritz 2009). The amino acid production from the thermodynamic calculation was too much lower than those obtained through my previous laboratory experiments (Elmasry et al. 2021). It probably due to the hydrolysis step in the laboratory

analysis that liberates large amounts of amino acids, while the calculation treated the formation of free amino acids only, not amino acid precursors.

Vinogradoff et al. (2020b) conducted hydrothermal experiments with a starting solution containing hexamethylenetetramine (HMT) heated for 2 d at 150 °C. Although the yields of amino acid in my calculation was very low, their results are consistent with my results since Gly was the dominant amino acid in both studies. In addition, after increasing the experimental duration to 7 d, their results (Vinogradoff et al. 2020b) showed that Gly increased significantly to 190.6 μM, still, the dominant amino acid, which also consistent with my 7 d calculation results, since Gly keep increasing in the model simulation without minerals.

In the FAW without minerals, the concentration of Gly continuously increased at 150 °C with time. Many studies have revealed that α -amino acids are abundant in the Murchison meteorite (Botta et al. 2007; Glavin et al. 2010), as well as through hydrothermal synthesis experiments formed from a gas mixture of methane and nitrogen with the principal metal ions present in seawater (Yanagawa and Kobayashi 1992), and a solution containing carbonate, cyanide and formaldehyde (Marshall 1994) and a gas mixture of carbon monoxide, ammonia, and water (Elmasry et al. 2020), and from a solution mixture of formaldehyde and ammonia in presence of water at short heating duration (Elmasry et al. 2021). However, the concentration of amino acids decreased with increasing the temperature more than 150 °C.

The presence of minerals; olivine, montmorillonite, and serpentine enhanced the Gly yield at a short heating duration (1 d). Moreover, montmorillonite could enhance Gly production after heating for 3 d. My calculation results are supported by previous experimental studies that demonstrated that montmorillonite enhanced the yield of amino acids in prebiotic conditions simulating early Earth (Shimoyama et al. 1978; Hashizume 2012). Montmorillonites have exceptionally high surface areas due to their layered structure, which increases their ability to adsorb water and organics to their surface (Cleaves et al. 2012). As consequence, amino acid preservation is probably promoted by montmorillonite due to its high surface areas and small pore sizes (Dos Santos et al., 2016).

However, after 7 d, the formation of the amino acids decreased significantly with all silicates; thus, the decomposition of amino acids could be enhanced by phyllosilicates and olivine at longer heating durations. Despite this, while in the presence of olivine, the Gly concentration was still having some stability behavior with time, since its concentration at 1 d was almost as same as after 7 d. Overall, it was suggested from my calculation results that minerals enhanced the production of amino acids at short heating durations yet enhanced their decomposition for longer heating durations.

The temperature could affect the production of amino acids. With increasing the temperature (25 °C–150 °C), the yields of amino acid increased in the FAW model without minerals as well as FAW model with phyllosilicates, followed by a decrease in their production from 150 °C to 300 °C. However, the presence of olivine enhanced the yields of amino acids with increasing the temperature until 300°C. It should note that the thermodynamic databases used for this study are only applicable for investigations up to 300°C.

Meteoritic organic matter is closely correlated with phyllosilicates, proposing that these minerals may have had catalytic effects during molecular evolution in the early solar system (Pearson et al., 2002). The production of the amino acids could be affected by the unique characteristics of phyllosilicates (Vinogradoff et al. 2020a; 2020b), such as structure, chemical composition, and crystal size. In my simulation at 150 °C and an alkaline pH, using formaldehyde and ammonia, the presence of montmorillonite induced the enhancement of amino acid production at 1 d and the decomposition of amino acids after longer heating. This could be due to the changes in the structure of montmorillonite during heating.

4.6. Conclusions

Numerical calculations showed that various kinds of amino acids could be formed from a mixture of formaldehyde, ammonia in the presence of water simulating aqueous alteration in small bodies in the Solar System. Minerals may play significant roles in the formation of organics, thus I added three kinds of minerals in the input data to evaluate their enhancing and inhibiting effects on amino acid production. Without minerals, most of the amino acids have the same behavior in response to temperature, hence they increased until ~150 °C, then starting to decrease. Olivine enhanced the yield of amino acids, whereas phyllosilicates enhance their decomposition. Gly concentration was enhanced without minerals in response to heating duration. Phyllosilicates (montmorillonite and serpentine) enhanced the decomposition of Gly, while olivine showed stability behavior with time. The concentration of the obtained amino acids was very low in comparison to the concentration obtained from the experimental analysis probably due to lack of the hydrolysis step that liberates large amounts of amino acids, thus free amino acids only could be formed, not amino acid precursors. Therefore, I considered that the factors that affect the amino acid production are the concentrations and the molar ratios of the starting solution (NH₃ and HCHO), pH, CO₂ and CO concentration, formate and acetate concentrations, temperature, heating time, presence of minerals, and the different kinds of minerals.

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Chapter 5

General conclusions and Future plan

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5.1. General Conclusions

Although life on Earth probably began between 3.5 and 3.9 billion years ago, the origin of life is still one of the great mysteries in the Universe. The early Solar System comprised a broad area of abiotically created organic compounds, including interstellar organics which were integrated into parent bodies of meteorites, and eventually delivered to the early Earth. Delivery of the organic compounds including amino acids in meteorites to the early Earth may have been important for the origin of life. Indeed, some meteorites called “carbonaceous chondrites” are known to contain abiotically synthesized amino acids and other organic compounds. The aim of this thesis research is to evaluate the stability of simulated interstellar complex organic compounds under high temperature and pressure conditions simulating parent bodies of meteorites. Most chondrite parent bodies accreted water ice together with anhydrous minerals and subsequently experienced aqueous/hydrothermal alteration when the ice melted in response to heating, mostly from radioactive decay of ^{26}Al .

In addition to the amino acid precursor formations in interstellar environments, it is known that the amino acids could have been synthesized in the meteorite parent bodies during hydrothermal alteration. Minerals could play an important role in the evolution of meteoritic organic matter, but the effects of minerals on the synthesis of organic matter in parent bodies are not well known. Thus, the roles of minerals on the amino acid productions were evaluated in environments simulating hydrothermal alteration in parent bodies of meteorites. This thesis contains five chapters and abstracts of each chapters are described below.

Chapter 1 describes the sources of the organic matter in early Earth, endogenous supply and exogenous delivery and their impacts on the origin of life in the light of the previous researches. I focused on the importance of delivery of organic matter, including amino acids, in meteorites to the early Earth in the origin of life. Carbonaceous chondrites, which are the most primitive known meteorites, are rich in organic matter. These organic compounds are divided into insoluble complex macromolecular organic matter (more than 70%) and soluble organic matter (less than 25%), which provides a chemical record of prebiotic chemical evolution that occurred before and during the formation of early solar system. Organic matter could be formed through various scenarios, including aqueous alteration or a hydrothermal reaction in the meteorite parent bodies. Minerals could affect hydrothermal reactions of organic matter, leading to catalyzing/inhibition effects depending on its nature and structure.

Chapter 2 deals with possible hydrothermal alteration and stabilities of complex macromolecular amino acid precursors under high temperature and pressure conditions

simulating parent bodies of meteorites by using an autoclave. It was considered that at the first stage of chemical evolution, the main compounds formed abiotically are complex organic compounds with high molecular weights. Therefore, simulated interstellar complex organic compounds were synthesized by proton irradiation of a gas mixture of CO, NH₃, and H₂O, which were known to release amino acids after acid hydrolysis, and their stability were tested during hydrothermal alteration simulating meteorites parent bodies under elevated temperature. These results suggested that the interstellar complex organic analog could maintain as amino acid precursors after being treated at high temperature and pressure. However, the molecular structures were altered during heating to form organic compounds that are more stable and can survive in elevated hydrothermal conditions.

Chapter 3 deals with abiotic synthesis of organic matter in aqueous environments simulating parent bodies of meteorites and the effects of minerals on the production of amino acids. Therefore, planetesimal environments were simulated by using an aqueous mixture of H₂CO and NH₃ in the presence of water, which produced amino acids after acid hydrolysis. Moreover, minerals were added to the previous mixture to examine their catalyzing/inhibiting impact on amino acid formations at 150 °C under different heating durations. These results indicated that minerals affected the formation of amino acids in aqueous environments in small Solar System bodies and that the amino acids could have different response behaviors according to different minerals.

Chapter 4 deals with the minerals' impacts on amino acid production in environments simulating planetesimals thermodynamically by using Geochemist's Workbench 14.0 (GWB 15.0) software. This calculation was supported by laboratory experiments to constrain the hydrothermal reaction conditions on parent bodies of meteorites. The input starting materials in the program were formaldehyde, ammonia, and water, in a molar ratio of 9 : 1 : 100 under alkaline conditions (pH 10) at temperatures (25 °C to 300 °C), with and without different kinds of minerals (10 g/L). Various amino acids have been formed with the predominance of Gly, and their concentrations keeping increase till about 150 °C, then started to decrease. Gly concentration was enhanced to decompose after 7 d with phyllosilicates, while olivine exhibited some stability behavior over time. These results showed that the amino acids could have different response behaviors based on the presence of different minerals. Further simulation experiments are needed to be conducted to examine the effect of different chemical parameters, e.g., temperature and pH, on the stability of the amino acids and the alteration of minerals.

We expect that the thermodynamic calculation can provide long-term prediction since the parent body process proceeded at a lower temperature, possibly close to 0 °C up to 150 °C. with much longer duration, could be millions of years, while such environments cannot be simulated in laboratory experiments. In summary, I hope that my experiments have effectively contributed to a better understanding of the relationship between minerals and the production of amino acid production in aqueous environments in small Solar System bodies.

5.2. Future Plan

Despite ongoing research, the effects of minerals on amino acid production are still well unknown, and further research is required. As a result, I will expand and develop my current research to offer a rich and detailed scenario of the mineral-amino acid relationship. My strategy will be divided into two stages.

In the first stage, the both strategies of numerical calculation and experimental analysis will be expanded for further interpretation the impact of the minerals on the production of the amino acids in the early solar system. Laboratory experiments will be performed by heating aqueous solutions of formaldehyde and ammonia with minerals (montmorillonite, olivine, serpentine, etc.) at 50 °C to 150 °C for days to weeks, followed by amino acid analysis using HPLC. In addition, the numerical calculation based on the thermodynamic calculations will be conducted using the Geochemist's Workbench software (GWB 15) under more various conditions than experiments, including starting solution composition, temperature, heating duration, and pH.

In the second stage, I will evaluate the impact of minerals on amino acid synthesis in real meteorites powders, which I already have, namely, Allende, Murchison, and MIL090001 (CR2). Comparing the results of the real meteorites with those of the above-mentioned laboratory experiments would provide a reasonable opportunity to validate and optimize the starting materials for the simulation experiments to yield more amino acids, allowing me to expand my research.