

Hydrothermal biochemistry: from formaldehyde to oligopeptides

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Abstract Hydrothermal reactions of formaldehyde (CH_2O), which is mainly formed by CO_2 , are considered to be of most importance in the abiotic synthesis of complex organic molecules in chemical evolution on the primitive Earth. We show the hydrothermal formation of amino acids and oligopeptides in neutral or weak acidic solutions from CH_2O in the presence of metals and metal oxides. In these hydrothermal processes, metal or metal oxides selectively catalyzed a series of hydrothermal reactions from CH_2O to amino acids, whereas the further reactions for the formation of oligopeptides proceeded without any catalyst. Our experimental observation may provide a possible clue to understanding the initial chemical evolution in Earth's primitive atmosphere of the origin of life.

Introduction

Exploring the origin of life is one of the most fascinating and inspiring scientific challenges in our age. The facts and hypotheses on this subject have revealed a considerable progress in both experimental and theoretical aspects, including the formation of amino acids in well-known electric discharge experiments in the environment of the atmospheric composition of CH_4 , NH_3 , H_2 , and H_2O [1] or CO_2 , N_2 , and H_2O [2]. The hyperthermophilic and chemotrophic interpretations for the origin of life in an

iron–sulfur world were proposed [3–8]. In 1977, the exploration of the sea-floor hot springs on the Galapagos mid-ocean rift indicated that microorganisms can survive under hydrothermal conditions. Corliss thus suggested a submarine hot spring hypothesis for the origin of life [9]. Agreement starts and stops with the fact that life on the earth has existed for at least 3.5 billion years and organic molecules were required for its emergence [10, 11], but it still a big puzzle how these organic molecules were formed and how they were made steady to form amino acids and peptides on the paths up to self-replicating systems of nucleotides and proteins.

It is convinced that abundant CH_2O molecules existed under primordial hydrothermal conditions, due to photochemical reactions of the atmosphere (N_2 , H_2O , CO_2 , and smaller amounts of reduced gases such as H_2 and CO) and hydrothermal reactions of CO_2 and carbonates in the primeval ocean, before life was originated [12, 13]. Under such weak reducing conditions, CH_2O in the atmosphere could be precipitated from the atmosphere into the early oceans, providing a source of organic carbon [14]. However, CO_2 and CH_2O , the simplest molecules, may play a key role in the prebiotic synthesis of complex organic molecules on the primitive earth [15, 16].

Our previous work shows that CH_2O molecule was able to be produced as an intermediate product in the process of phenol formation from the reaction of CO_2 and water in the presence of Fe powder [17]. We thus motivate further search for the subsequent reactions of CH_2O in the primeval water environment, which must have clear implications for a biotic synthesis of complex organic molecules in the origin of life. It was reported that sugars were produced by the formose reaction under basic conditions, but they were unlikely to contribute to chemical evolution on the primitive earth, because the reaction

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process is less selective and particularly inefficient [18–22]. With an aim towards understanding the pivotal and disputed initiative step, we concentrated the reactions of CH_2O in water in neutral solutions in the presence of metal and metal oxides as catalysts based on the well-established mild hydrothermal method [23].

Experimental

Hydrothermal reactions of CH_2O with H_2O and GC-MS characterization

We began with the hydrothermal experiments by carefully purifying the starting materials. In a typical experiment 2.22 mL (30 mM) solution of CH_2O was mixed with 25 mL H_2O , and 560 mg (10 mM) of Fe powder (10–20 μm in diameter) was added. Fe powder (FCC phase) was purified by standard calcinations, and water was redistilled to ensure free of the organic. The reaction mixture was loaded into a steel autoclave (Fe–Cr–Ni alloy, GB1220-92) with a filling capacity of 90% and allowed reactions at 120 °C for 9 days. The final pH value of 5 was measured in the reaction solution. ICP of the brown solution after reaction measured 1% Fe ions in weight, indicating the formation of Fe^{2+} by a redox reaction of Fe with water. GC-MS data were collected on the TRACE MS GC-MS (Finnigan Co.). Temperatures: column, 50 °C for 1 min, then increased from 50 to 150 °C at the speed of 20 °C/min. The final temperature is 150 °C. Sample volume: 1 μL . Column type: HP-5 ms. MS: EI source.

Hydrothermal formation of glycine

In a typical run, 7.58 mL (100 mM) solution of acetic acid was adjusted to pH 5, 6, 7 with the addition of 25% solution of ammonia, and then was stirred after adding 25 mL water, to which 160 mg (1 mM) Fe_2O_3 was added. Metal oxides were purified, and water was redistilled to ensure free of the organic. The reaction mixture was loaded into a steel autoclave (Fe–Cr–Ni alloy, GB1220-92) with a filling capacity of 90% and allowed reactions at 120 °C for 5 days. After the reaction the products were cooled and filtered, the laurel-green solution obtained was identified and quantified by HPLC.

Hydrothermal formation of racemic D, L-alanine and HPLC characterization

In a typical run, 7.46 mL (100 mM) solution of propionic acid was adjusted to pH 5, 6, 7 with the addition of 25%

solution of ammonia, and then was stirred after adding 25 mL water, to which 160 mg (1 mM) Fe_2O_3 was added. Metal oxides were purified, and water was redistilled to ensure free of the organic. The reaction mixture was loaded into a steel autoclave (Fe–Cr–Ni alloy, GB1220-92) with a filling capacity of 90% and allowed reactions at 120 °C for 5 days. After the reaction the products were cooled and filtered, the solution obtained was identified and quantified by HPLC. We examined the effect of Fe_2O_3 by the use of other metal oxides under the same conditions. Experiments were carried out at 120 °C and Sm_2O_3 , ZrOCl_2 , TiO_2 , Y_2O_3 , and CeO_2 were used. The racemic D, L-alanine was obtained at pH = 5 when ZrOCl_2 was used as catalyst, whereas no amino acids were found when Sm_2O_3 , TiO_2 , Co_2O_3 , Y_2O_3 , and CeO_2 existed as catalyst. The characterization was performed on the HPLC (Agilent 1100), using the columns Agilent Zorbax Eclipse XDB-C18, 4.6×20 5 μm and ultraviolet detector (360 nm). Column oven was set at 40 °C. Gradient elution: Flow rate: 1 mL/min;

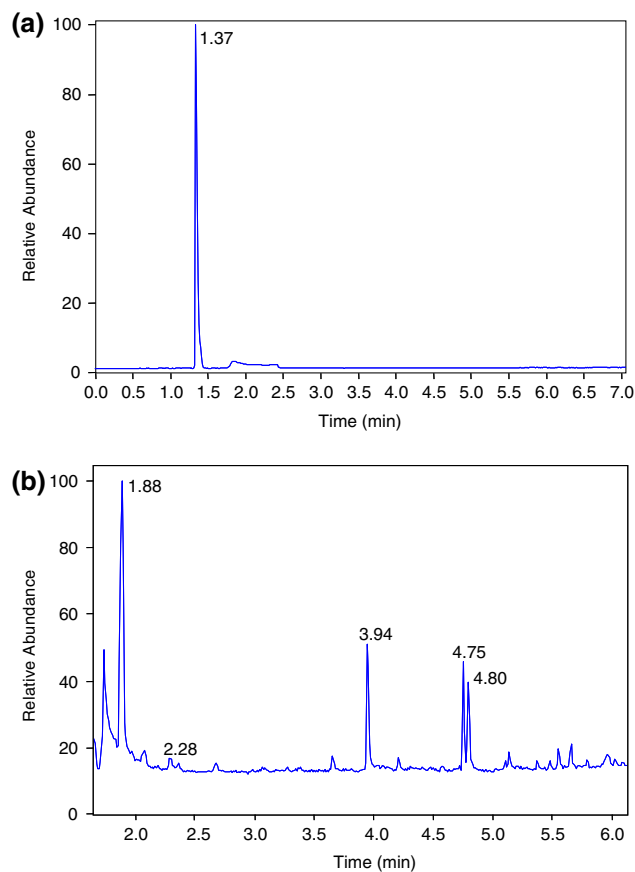


Fig. 1 (a) GC spectrum of the products of formaldehyde with water without catalyst under hydrothermal conditions. $t_R = 1.37$, formaldehyde; (b) GC spectrum of the products of formaldehyde with water in the presence of Fe powder under hydrothermal conditions. $t_R = 1.72$, formic acid; $t_R = 1.88$, acetic acid; $t_R = 2.28$, propionic acid; $t_R = 3.94$, propyl propionate; $t_R = 4.75$, methyl acetate; $t_R = 4.8$, propyl isobutyrate

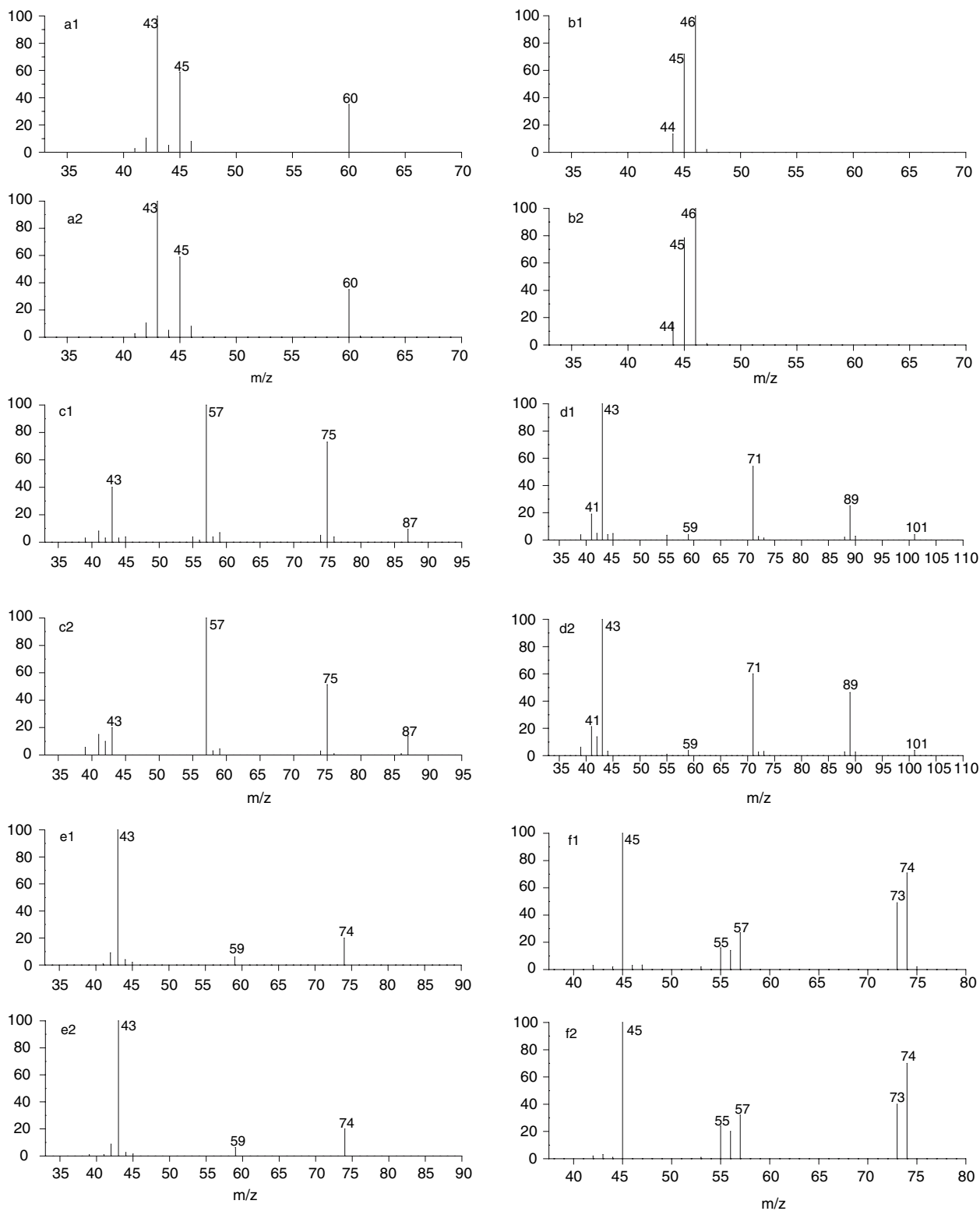


Fig. 2 MS diagrams for comparison of the products with standard samples in MS database-NIST. **(a)** Acetic acid, **(b)** formic acid, **(c)** propyl propionate, **(d)** propyl isobutyrate, **(e)** methyl acetate, **(f)** propionic acid. 1 is mass spectrum of the products; 2 is mass spectrum of standard samples. MS data were collected on the TRACE MS

GC-MS (Finnigan Co.). Temperatures: column, 50 °C for 1 min, then increased from 50 to 150 °C at the speed of 20 °C/min, final temperature 150 °C. Sample volume: 1 µl. Column type: HP-5 ms. MS: EI source

A: Acetonitrile: H₂O = 55:45 (v/v); B: 40 mM KH₂PO₄ (pH = 7.2). Time (min)/B%: 0/0, 1/0, 4/14, 10/40, 20/80, 21/100, and 21.3/0. Derivative method: In a run, a 2.5 mL autosampler vial was charged with 30 μL of sample, 30 μL 0.5 M NaHCO₃ (pH = 9.0) and 10 μL derivatization reagent (1% 2, 4-dinitro fluorobenzene in acetonitrile) sealed with a screw cap. This reaction proceeded at 60 °C for 1 h. After the reaction 0.5 mL 40 mM KH₂PO₄ (pH = 7.2) was added in and 20 μL mixed solution was injected onto the column.

Hydrothermal formation of peptides and LC-MS/MS characterization

In a typical run, 10 mM glycine or racemic D, L-alanine (purchased by Fluka) was dissolved with a little distilled water. The solution was adjusted to pH 2.5 by hydrochloric acid through stirring. The reaction mixture was loaded into a steel autoclave (Fe–Cr–Ni alloy, GB1220-92) with a filling capacity of 90% and was allowed reactions at 120 °C for 9 days. Then the solution was identified by LC-MS/MS and dipeptides were detected. The LC-MS/MS system consists of an Agilent 1100 series (Agilent Technologies, Palo Alto, CA, USA) binary pump, autosampler, and Applied Biosystems Sciex API 4000 mass spectrometer (Applied Biosystems Sciex, Ontario, Canada) using electrospray ionization (ESI). The Hypersil CN column (5 μm, 150 × 4.6 mm I.D. from Dalian Elite Separation Science and Technology Corp, Dalian, China) was used for the detection of diglycine with the mobile phase consisting of methanol-1% formic acid (75:25 v/v) delivered at the speed of 1 mL/min at 30 °C. The Agilent Zorbax AQ C18 column (5 μm, 150 × 4.6 mm I.D. from Agilent Technologies, Palo Alto, CA, USA) was used for the detection of dialanine with the mobile phase consisting of methanol-1% formic acid (3:97 v/v) delivered at the speed of 1 mL/min at 30 °C. ESI was operated in the positive ion mode with nitrogen as the nebulizer, heater and curtain gas. High-flow gas flow parameters were optimized by making successive flow injection while introducing mobile phase into the ionization source at the speed of 1 mL/min. Optimum values for the nebulizer, heater and curtain gas flow rates were 55, 40, and 30 units, respectively. The ionspray needle voltage and heater gas temperature were set at 5,100 V and 500 °C, respectively. Response was optimized by syringe pump infusion (5 μL/min of a solution in the mobile phase containing analytes into the stream of mobile phase eluting from the column). The pause time was set at 5 ms and the dwell time at 400 ms. The detector was operated at unit resolution in the multiple-reaction monitoring mode using the transition of the protonated molecular ions of Gly–gly at m/z 133.0 → 76.0 and

Ala–ala at m/z 161.1 → 90.0. The collision gas (N₂) was set at 3 units and collision energies of 14 eV were used for both analytes. The instrument was interfaced to a computer running Applied Biosystems Analyst version 1.3 software.

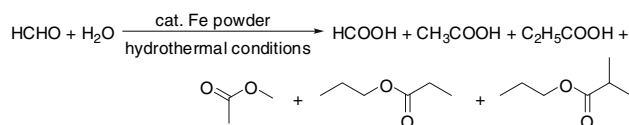
Results and discussion

Hydrothermal reactions of formaldehyde

We conducted the hydrothermal reactions of purified CH₂O in water at 60–160 °C in the presence of Fe powder. Surprisingly, we found rich products as detected by GC. Although these formed complicated organic compounds (see GC in Fig. 1b) have not been wholly identified at present, at least six main simple molecules were clearly identified by mass spectroscopy (MS) (Fig. 2), which are formic acid (HCOOH), acetic acid (CH₃COOH), propionic acid (C₂H₅COOH), methyl acetate (C₃H₆O₂), propyl propionate (C₆H₁₂O₂), and propyl isobutyrate (C₇H₁₄O₂). The selected reactions of CH₂O under hydrothermal condition were summarized in Scheme 1.

We have examined the effect of Fe by the use of other metals or zeolites. The best catalyst is Fe powder which efficiently leads to considerable amounts of organic compounds in the first step of reactions. The background experiments without any catalyst did not result in products as monitored by gas chromatography (GC) (see Fig. 1a). The catalyzing role of Fe may be in accordance with its most abundant existence in our earth. This also reflects the relatively large redox potential of Fe (−0.44).

In order to maximize the yields of the main products, we carried out kinetic experiments for the selected four products. Kinetic curves for the formation reactions of CH₃COOH, C₃H₆O₂, C₆H₁₂O₂, and C₇H₁₄O₂ are shown in Fig. 3a. The yields (0.5–2.5 Mol%) of products increase with time in the reaction process. We examined the influence of filling capacity of reactor, which is related to the pressure of reaction, on the yields of the products. Yields increase slightly with filling capacity as shown in Fig. 3b, which proved pressure benefiting the yield. Our experiments evidenced the fact that the hydrothermal reactions of CH₂O in the presence of Fe powder could be enhanced with time and pressure, which accordingly more or less



Scheme 1 Reactions of HCHO and H₂O in the presence of Fe powder

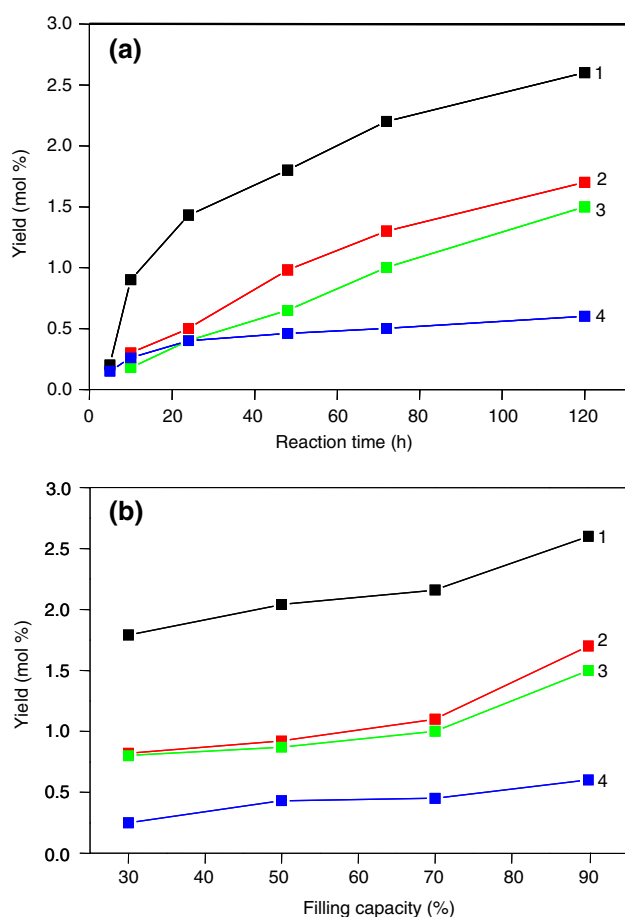
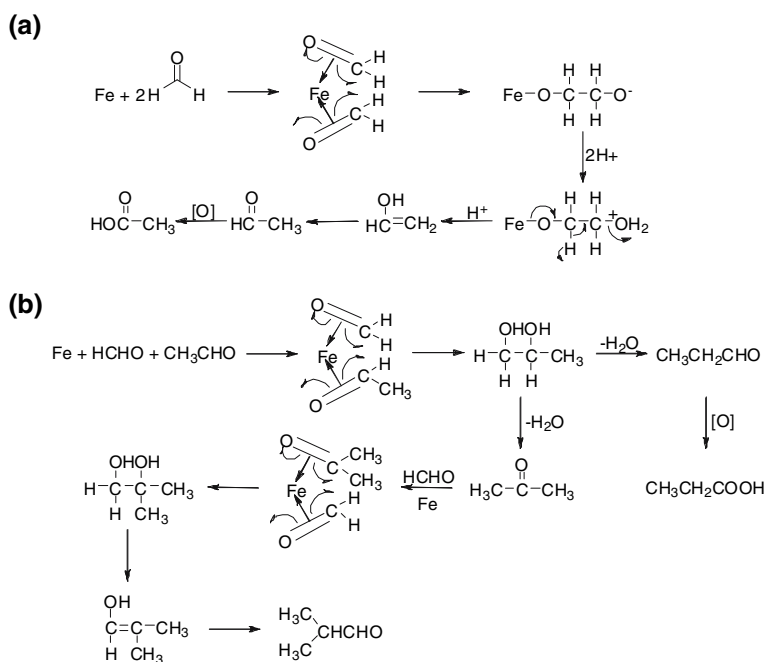


Fig. 3 (a) Yield dependence of reactor filling capacity and (b) kinetic curves of formation of acetic acid (1), propyl propionate (2), propyl isobutyrate (3), and methyl acetate (4)

Scheme 2 Possible mechanisms for the formation of acetic acid (a), propionaldehyde, and isobutyraldehyde (b)



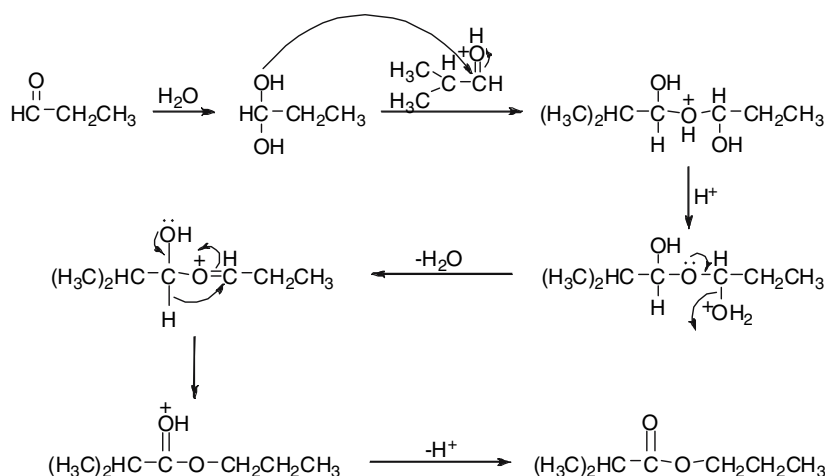
implies the possible chemical processes in deep sea at geographic ages.

In our neutral solutions we did not see any product from the so-called formose reaction which generally gives the racemic mixtures of straight-chain and branched-chain aldoses and ketoses with C_{2-7} or perhaps even higher species [18–20]. Apparently, the mechanisms of our hydrothermal reactions are quite different from those of the formose reaction. Owing to our simple hydrothermal system where only CH_2O and Fe existed, it is reasonable to deduce the possible mechanisms for the formation of acetic acid by the participation of Fe in the oxidation of acetaldehyde (Scheme 2a). Sequentially, Fe coordinates with an acetaldehyde molecule and a formaldehyde molecule to produce propionaldehyde and isobutyraldehyde (Scheme 2b). Acetaldehyde and formaldehyde molecules further react to give methyl acetate and two propionaldehyde molecules react to each other to form propyl propionate. Finally isobutyraldehyde reacts with propionaldehyde to produce propyl isobutyrate (Scheme 3).

Amino acids

We continued our experiments with ammonia into the reaction systems. During the reaction of ammonia with acetic acid or propionic acid, we witnessed the formation of two amino acids, e.g., glycine and racemic D, L-alanine in the presence of metal oxides (Scheme 4), as indicated by high pressure liquid chromatography (HPLC) spectra

Scheme 3 Possible mechanisms for the formation of propyl isobutyrate



(Fig. 4). Generally, in such nucleophilic reactions the products are not amino acids. Our hydrothermal reactions are obviously extraordinary. More interestingly, rather than other metal oxides, iron oxide- Fe_2O_3 is the most effective catalyst to reach the maximal yields of amino acids in these reactions. As we recall from the above CH_2O hydrothermal reactions, Fe as a catalyst was also more effective than other metals under the same conditions and in the solution we indeed detected the existence of Fe^{2+} ions by inductively coupled plasma (ICP). The catalysis of Fe, Fe^{2+} , and Fe_2O_3 in the previous reactions is corresponding to their biotic catalysis. We therefore experimentally evidenced that Fe and its oxide as crucial biotic catalysts play an important role in our series of hydrothermal reactions from CH_2O to amino acids.

Water molecule serves as a lubricant or a medium for biogeochemical cycling. Since, such a superb polarity solvent itself is in constant flux, it helps to convey many substances hither and thither, and makes them react to each other in hydrothermal reaction systems.

Oligopeptides

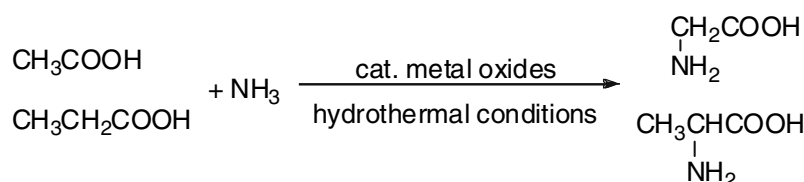
The formation of the racemic amino acids gives us an impetus to examine if chirality can be stabilized in the formation of peptide in hydrothermal systems. We thus carried out the synthesis of peptide from the racemic amino acids based on our previous experiments [24, 25]. Firstly, it is very interesting that oligopeptides were formed here without any catalyst and secondly, the yield of DD- or

LL-dialanine is higher than that of DL- or LD-dialanine (Fig. 5). In the first stage of the formation reaction of oligopeptides from L-alanine, the ratio of the yields of DD- or LL-dialanine to DL- or LD-dialanine is ca. 8. With the increasing reaction time, the ratio decreases to ca. 2 (Fig. 6). The racemization occurred during the formation of oligopeptides in hydrothermal systems and this process was dynamic.

It clearly showed that the formation of DD- or LL-dialanine is thermodynamically controlled, whereas the formation of DL- or LD-dialanine is kinetically controlled. It is a kind of unbalance of enantiomers. We need to know in the future study if the racemization is derived from the original amino acid before the coupling reaction or directly from the coupling reaction. Further investigation is necessary to clarify each content of the enantiomers formed. However, this observation gives us a plausible explanation for the formation of these biopolymers in hydrothermal systems, and helps in understanding the origin of homochirality in the chemical evolution of early life.

As a systematic study, we tried to illustrate the possible process from CO_2 to peptides or even more complicated biomolecules. The mechanisms for the hydrothermal reactions from CH_2O to diglycine based on the experimental facts were proposed as shown in Fig. 7. The reaction may start on the surface of Fe powder, where oxygen atoms of two formaldehyde molecules coordinate with the surface Fe atoms of Fe powder to produce acetic acid through a series of reactions. The acetic acid reacts to ammonia on the surface of Fe_2O_3 to form glycine (Fig. 7b), and finally, two glycine molecules couple each other to form diglycine by dehydration.

Scheme 4 Reactions of acetic acid and propionic acid with ammonia in the presence of metal oxides



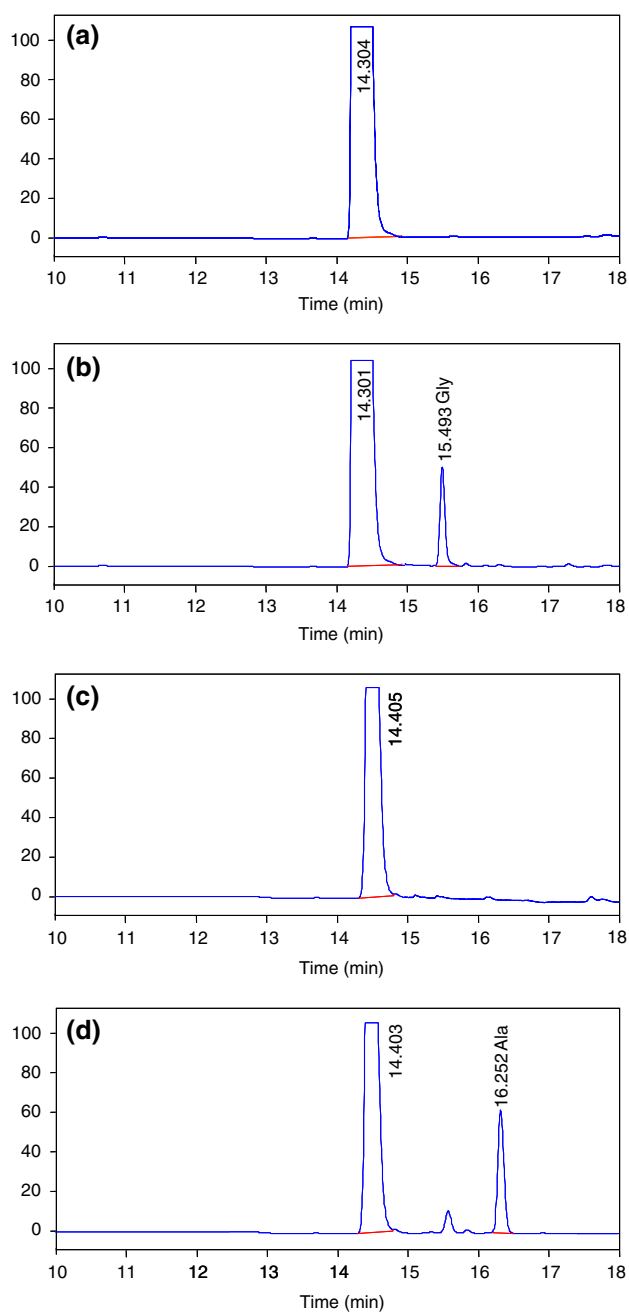


Fig. 4 HPLC spectra of the products of the reactions of acetic acid with ammonia under hydrothermal conditions. (a) no catalyst, $t_R = 14.304$, derivative reagent, (b) $t_R = 14.301$, derivative reagent; $t_R = 15.493$, glycine. HPLC spectra of the products of the reactions of the propionic acid with ammonia under hydrothermal conditions, (c) no catalyst, $t_R = 14.405$, derivative reagent, (d) $t_R = 14.403$, derivative reagent; $t_R = 16.252$, racemic D, L-alanine

Conclusions

Our experiments provide a clue to solving the long-disputed problem in the hydrothermal origin of life as many scientists suggested [26–30], e.g., not only sugars but also amino acids and oligopeptides were able to be formed from

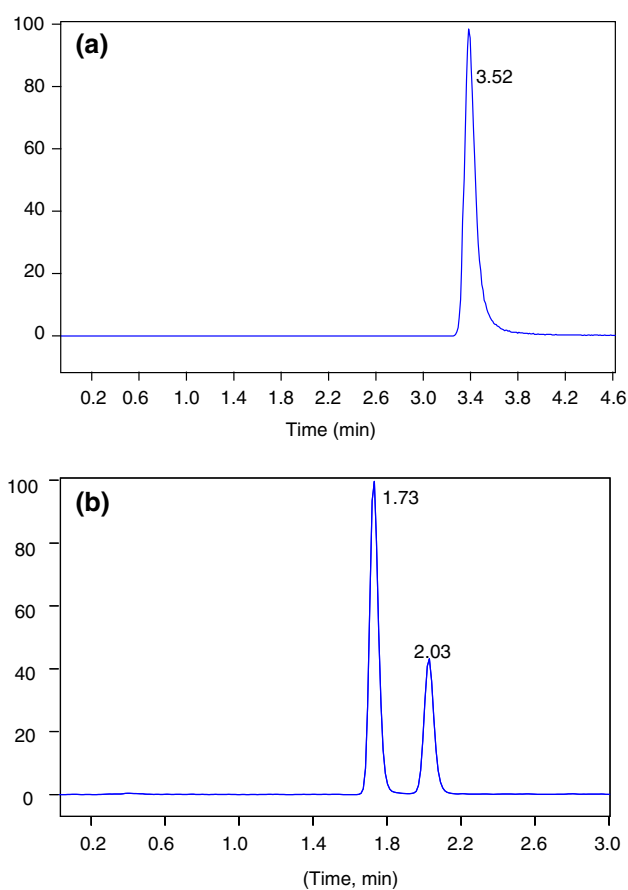


Fig. 5 HPLC spectra of the products of glycine (a) and racemic D, L-alanine (b). Diglycine at $t_R = 3.52$ (a), DD- or LL-alanine at $t_R = 1.73$ and DL- or LD-dialanine at $t_R = 2.03$ (b)

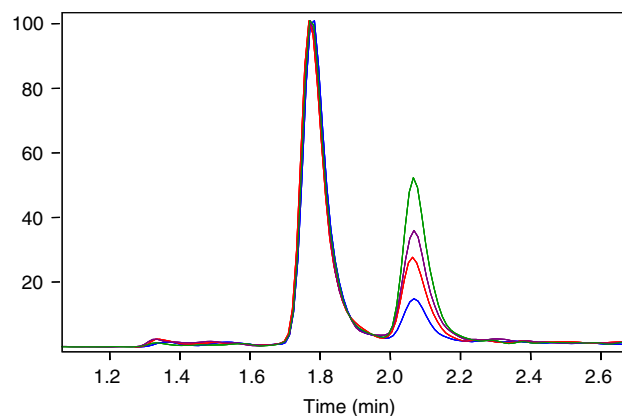


Fig. 6 HPLC spectra of the dialanine systems at different reaction time for 1 day (blue line), 4 days (red line), 7 days (violet line), and 9 days (green line)

the prebiotic molecule- CH_2O . Obviously, amino acids and oligopeptides are significant substances in the process of biotic chemical evolution. Moreover, the products of CH_2O hydrothermal reaction are relatively stable, and thus

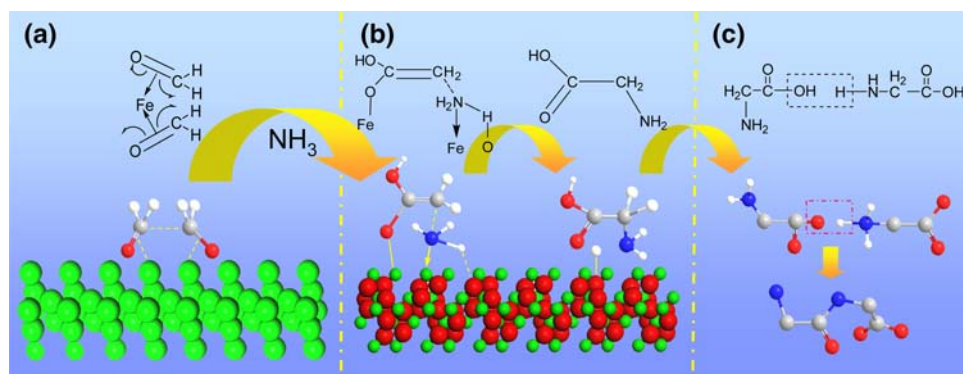


Fig. 7 Schematic illustration of the proposed mechanisms for the hydrothermal reactions from CH₂O to diglycine. This process begins with the coordination of formaldehyde molecules with Fe atoms to produce acetic acid (a). The formed acetic acid reacts to ammonia on the surface of Fe₂O₃ to form glycine, where hydrogen atom of

ammonia coordinates with oxygen atom of Fe₂O₃, leaving a hydroxyl group on the surface (b). Finally, two glycine molecules couple each other to form diglycine (c). Fe: green ball; O: red ball; oxygen, H: white ball, C: grey ball, and N: blue ball

possible subsequent reactions towards more complicated bio-molecules may proceed. Since we have accomplished the transformation from single-carbon molecule to oligopeptides through a series of hydrothermal reactions on the surface of metal and metal oxides, we believe that the synthesis of other amino acids, which are necessary to the life, based on single-carbon sources, such as CH₂O, CO, CO₂, etc. should be possible. As more complicated subsequent reactions occurred with time, the formed biotic organic molecules may provide molecular blocks to produce nucleic acids and proteins with biological functions of self-replication and catalysis, which are further study in the field of hydrothermal chemistry.

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