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Hydrothermal treatment of glycine and adiabatic expansion cooling: implications for prebiotic synthesis of biopolymers

Yasuhiro Futamura · Kouki Fujioka · Kenji Yamamoto

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Abstract There have been several studies on biopolymer synthesis under hydrothermal conditions. The conventional hydrothermal methods make it possible to synthesize only a dipeptide and short oligopeptides as well as cyclo-dimer, from amino acids. As these studies that were applied with various quenching methods suggested the importance of quenching rate from hydrothermal conditions, rapid quenching could avoid hydrolysis of the oligomers that had already been synthesized under hydrothermal conditions. In this study, therefore, we designed a novel hydrothermal flow reactor adopted with adiabatic expansion cooling system from the reason that it was thought to be one of the most rapid quenching methods. It mimics geysers, fumaroles, hot springs, and volcanic eruptions. Once aqueous solutions of monomers were treated at high temperature and pressure, the solutions were released into the atmosphere through an orifice to be depressurized and cooled down simultaneously with the Joule–Thomson effect. We demonstrated oligomerization of glycine up to decamer (Gly_{10}) by using the flow reactor, which had never been yielded with any other quenching methods. This suggests that rapid quenching methods under non-equilibrium conditions such as adiabatic expansion cooling is an efficient way to produce long oligomers connected by covalent bonds via dehydration condensation.

Y. Futamura · K. Fujioka · K. Yamamoto (✉)
International Clinical Research Center, Research Institute, International Medical Center of Japan, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan
e-mail: backen@ri.imcj.go.jp

Y. Futamura
Department of Bioactive Molecules, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan

K. Yamamoto
Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, 2-1-1 Katahira, Aoba-ku, Sendai 980-8577, Japan

Introduction

Origins of life and prebiotic synthesis of its component

On the basis of phylogenetic analyses, hyperthermophiles are located near the root of the phylogenetic tree. From the view of origins of life, the last common ancestor is thought to be a hyperthermophile [1]. In the context, the first living cell might have been born under hydrothermal conditions on the earth. However, where and how were the components of living cells synthesized?

Living cells are composed with water, inorganics, and organics such as proteins, nucleic acids, polysaccharides, and so on. Such organics are composed with monomer of amino acids, nucleotides, sugars, respectively (Fig. 1). The onset of polymerization must have been a major step in the chemical evolution that formed the precursors of life. In this paper, we'd like to focus on polymer synthesis from monomers under hydrothermal conditions.

Prebiotic synthesis of biopolymers in deep sea or in geysers?

Submarine "Alvin" discovered chemoautotrophic communities in a hydrothermal vent in deep sea of Galapagos Rift in 1977 [2, 3]. The temperature would be 100–400 °C and the pressure should depend on the hydraulic pressure

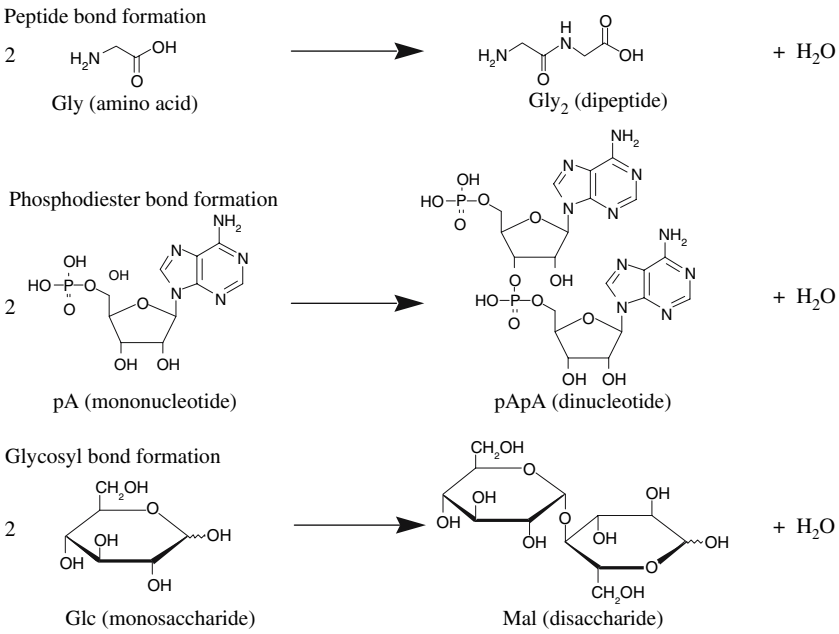

Fig. 1 Dehydration condensation of various monomers: amino acids, nucleotides, and saccharides

that is 25 MPa at a depth of 2,500 m. After then, hydrothermal vents are thought to be one of the candidates of place of origins of life and the components of living cells would be also synthesized from monomers there [4].

Imai and his colleagues tried to polymerize an amino acid using a flow reactor that simulates a submarine hydrothermal vent. As the results, up to trimer of oligoglycine were yielded without any catalyst added [4–7].

Now, we report a novel flow reactor dealing with subcritical and supercritical water for synthesizing oligopeptides from amino acids. The adiabatic expansion method for quenching and depressurizing the system was adopted to reduce the hydrolysis reaction of products. In almost all the previous studies the temperature and pressure of the system or the reaction time are often focused on. On the other hand, there is hardly any knowledge of methods of quenching and depressurizing the system. We considered these processes important.

In this study we worked on the reaction where glycine, the simplest amino acid, is formed into oligoglycines. After heating and pressurizing the glycine aqueous solution without any catalysts, it was quenched and depressurized with the adiabatic expansion method. The diglycine was produced about 45 times as much as in a previous study using water-cooled method for quenching the solution. It suggests that when the oligomer produced in water at high temperature and under high pressure was quenched, it was not hydrolyzed because of the higher rate of quenching. What is more, the concentration of linear-dimer was more than that of cyclo-dimer. It suggests our method was more suitable to make long peptides from amino acids. Actually we obtained longer chains of peptides up to decamer, which were not obtained in the previous studies.

The flow reactor in the previous studies simulated submarine hydrothermal vents in deep sea. On the other hand, the flow reactor with adiabatic expansion cooling simulates geysers and hot springs. Such a rapid quenching environment would be suitable for synthesis of hydrolytic compounds such as biopolymers.

Experimental details

The flow diagram of an experimental apparatus we designed is shown in Fig. 2. The apparatus had two reservoirs, one was for water and the other was for sample solution. Water was pressurized by the pump P-1 and was heated with the pre-heating unit. On the other hand, the pump P-2 pressurized sample solution. In order to raise the temperature of sample solution rapidly, sample solution and heated water were mixed together in the interflow block whose temperature was monitored with the thermoelectric couple TI-1. The needle valve made it feasible that the solution was quenched and depressurized more rapidly than by a water-cooling unit, a conventional method. Therefore it was expected that the hydrolysis or the decomposition of products would be suppressed during quenching and depressurizing the solution.

HPLC and LC-Mass analyses of oligoglycines were performed as described in Fig. 3.

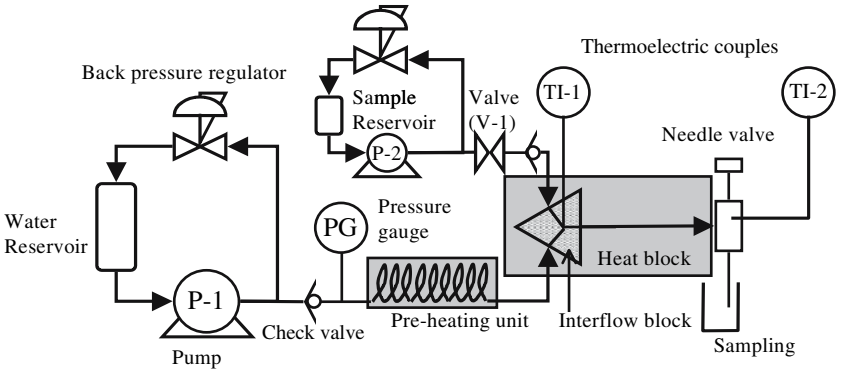

Fig. 2 The experimental apparatus adopted for an adiabatic expansion cooling with a needle valve to reduce the hydrolysis reaction of products; This flow reactor was designed to endure up to 40 MPa and 500 °C. Almost all the parts that contacted with the solution were made of stainless steel SUS316. A stainless tube, which a heat block kept heated, was placed between an interflow block and an inlet of a needle valve. The volume of the tube was 1.5 mL. The gap of needle valve is about 10 mm^2

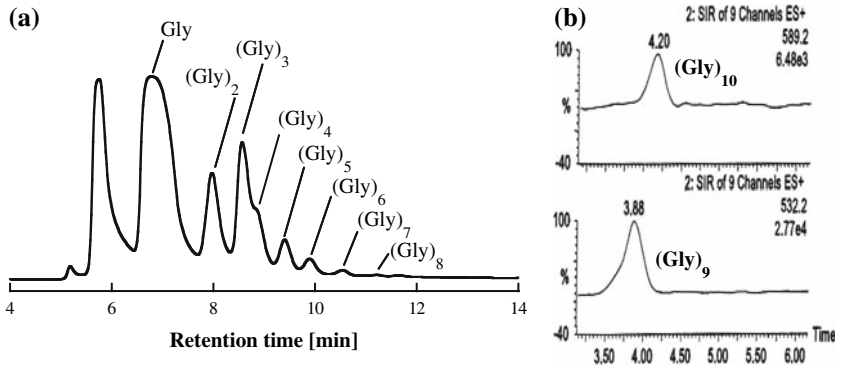

Fig. 3 (**a**) A high performance liquid chromatography (HPLC) profiles of the products in the sample that yielded from 0.1 mol/L glycine aqueous solution at 270 °C and 10 MPa and (**b**) Selected Ion Monitoring (SIM) mass chromatograms of decaglycine (top) and nonaglycine (bottom); $(Gly)_i$ denotes i-mer of glycine. The chromatograms were obtained from a Waters HPLC and LC/Mass spectrometry system respectively, with the use of a same reverse-phase column (Waters Xterra MS C_{18} 2.5 mm; 4.6 × 50 mm). Aqueous solution containing 50 mM KH_2PO_4 and 7.2 mM $C_6H_{13}SO_3Na$ (pH = 2.5) was used as a mobile phase in the condition for (**a**). The UV detector monitored the absorbance at 200 nm. In the condition for (**b**), aqueous solution of 1 mM $C_5F_{11}COOH$ was used as a mobile phase and the mass chromatograms of the oligoglycines were detected in Selected Ion Recording (SIR) mode

Results and discussion

Oligomerization of glycine

So far, there have been several studies on oligomerization of amino acids in subcritical water. Nevertheless, in most of the studies [4–7], oligomerization reaction did not occur efficiently.

Using the flow reactor with adiabatic expansion cooling (Fig. 2), after pressured, glycine aqueous solution was mixed into pre-heated water to make it reach the desired condition of concentration and temperature in the reactor, and it was quenched with adiabatic expansion. As shown in Fig. 3, we obtained various oligoglycines up to decaglycine (Gly_{10}) that had never been obtained with any other cooling methods [4–8].

Polymerization or circulation

It was found that the reaction solution contained diglycine and cyclo-diglycine (diketopiperazine) as its products. These concentrations were 0.078 and 0.015 mM respectively, which was calculated by HPLC chromatogram areas in Fig. 3. The concentration of the diglycine was 45 times as much as that in the previous study by Islam et al. [7]. Moreover, in comparing our study with the previous studies, we found an interesting fact about the ratios of linear-dimer against cyclo-dimer.

After the experiment of Imai et al. [4], not a few works on the polymerization of the amino acids has been done with the supercritical and subcritical water and supported the formation of cyclo-dimer. In all of these experiments, cyclo-dimer was detected more than the linear dimer [4–7].

Table 1 Ratio of diglycine to cyclo-diglycine after hydrothermal treatment of glycine

Reference	Reaction condition			Products conclusion
	P (MPa)	T (K)	Glycine (Raw material) (mM)	[cyclo-diglycine] / [linear-diglycine]
Imai et al. [4]	24	498	100	0.2
Ogata et al. [6]	24	523	100	0.17
Alargov et al. [5]	22	623	65	0.23
Islam et al. [7]	25	523	100	0.096
This study	24	523	100	5.2

Table 1 shows the results obtained from the different papers with their own devices under the different conditions. In all the previous studies, the ratios were less than one, but our result was opposite, the concentration of linear-dimer was more than that of cyclo-dimer. It meant that our experiment, especially adopting the process of depressurizing and quenching, was more suitable to make long peptides from amino acids because the seed of polymer was thought to be linear-multimer like diglycine. As a result, our apparatus using the needle valve had a possibility to be proper for making the hydrolytic compounds such as biopolymers.

The equilibrium reaction among glycine, diglycine, and cyclo-diglycine is shown in Fig. 4. Cyclo-dimer is formed via intramolecular condensation reaction of linear dimer, called unimolecular reaction, whereas linear oligomers are formed via intermolecular condensation of monomers and/or oligomers. Bimolecular reaction depends on the concentration of reactants. Adiabatic expansion cooling may contribute to increasing the concentration of reactants of bimolecular reaction.

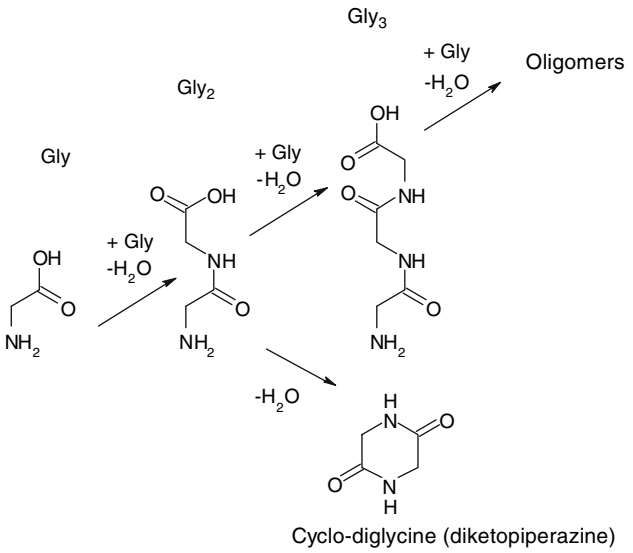

Fig. 4 Oligomerization of glycines and intramolecular condensation of diglycine

These results led us to the speculation that the amino acids could be polymerized linearly in the high pressure and high temperature water, but as for the cyclo-multimer, the polymerization would stop at the cyclo-dimer (Fig. 4). The generation of the cyclo-dimer from the monomer amino acids will compete the generation of the linear multimer. If the polymerization of the amino acids might be necessary for the initiation of the origin of life, this result leads us to a paradoxical problem.

Perspectives

Considering prebiotic chemical evolution, biopolymer syntheses have a common point: dehydration condensation of monomers. Dehydration condensation is a common reaction to form hydrolytic bonds between monomers, such as amide bond formation of oligopeptides, phosphoester bond formation of oligonucleotides, glycosyl bond formation of oligosaccharides, and acyl bond formation of lipids. Non-equilibrium cooling from hydrothermal conditions would be efficient for various types of dehydration condensation reaction.

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