HYDROTHERMAL SYNTHESIS OF OLIGOGLYCINES WITH ADIABATIC EXPANSION COOLING

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Abstract

There have been several studies on biopolymer synthesis under hydrothermal conditions. Although dimer and short oligomers were obtained from a monomer unit, few of longer oligomers were obtained. These studies that were applied with various quenching methods suggested the importance of quenching speed from hydrothermal conditions. We hypothesized a rapid quenching could avoid hydrolysis of the oligomers that had already been synthesized under hydrothermal conditions. We designed a novel hydrothermal flow reactor with adiabatic expansion cooling, which was thought to be one of the most rapid quenching methods. This system simulates geysers, fumaroles, hot springs and volcanic eruptions. After aqueous solutions of monomers were treated at high temperature and pressure, they were released into the atmosphere through an orifice to be depressurized and cooled down simultaneously with the Joule-Thomson effect. Using the flow reactor, we have demonstrated oligomerization of glycine up to decamer (Gly_{10}), 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.

Key words: prebiotic synthesis, hydrothermal system, dehydration condensation, biopolymer, peptide, adiabatic expansion, Joule-Thomson effect, quenching, non-equilibrium, subcritical water.

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Introduction

All of the biopolymers like proteins, nucleic acids and polysaccharides are composed of monomer units connected by covalent bonds via dehydration condensation. There have been several studies on biopolymer synthesis under hydrothermal conditions using various quenching methods; for example, pouring the fluid into cold water [1,2] or passing the fluid through a cooling tube [4,5]. These studies suggest the importance of quenching speed, because in the former studies longer peptides were obtained than in the latter, in spite of similar reaction conditions at high pressure and temperature.

Fig.1 shows the quenching pathway of the previous and this studies on phase diagram of water. In closed batch systems, the pressure and the temperature were changed gradually during cooling process (pattern A) [3]. In other previous works that simulates hydrothermal vents, the hydrothermal fluid was kept at high pressure during cooling and then was depressurized. The pathway was shown as pattern B [1,2,4,5].

Shock previously reported that the thermodynamic equilibrium of condensation/ hydrolysis reactions between glycine (Gly) and diglycine (Gly_2) would shift to the dehydration condensation as the temperature increased [6]. This report suggests that rapid quenching, that is non-equilibrium thermodynamics system, could avoid hydrolysis of the oligomers that had already been synthesized under hydrothermal conditions.

Now we designed a novel hydrothermal flow reactor with adiabatic expansion cooling, which was thought to be one of the most rapid quenching

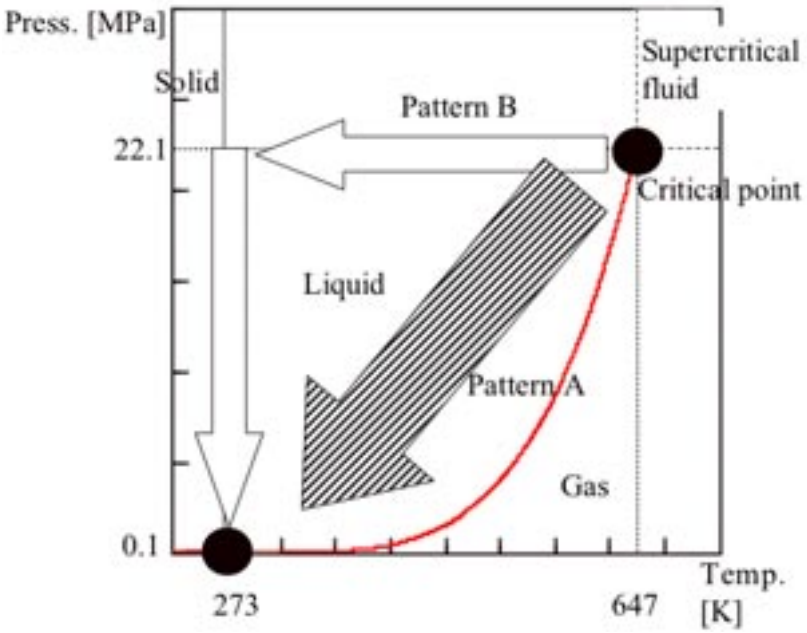

Fig. 1. Schematic quenching pathways on phase diagram of water. (A) Temperature decreases with pressure, and (B) Temperature decreases with keeping pressure.

methods [7]. After aqueous solutions of monomers were treated at high temperature and pressure, they were released into the atmosphere through an orifice to be depressurized and cooled down simultaneously with the Joule-Thomson effect shown as pattern A in Fig. 1 [8,9]. The Joule-Thomson effect is a process in which the temperature of a fluid is decreased below the Joule-Thomson inversion temperature by letting the fluid expand adiabatically. When a fluid suddenly expands from a high pressure to a low pressure, there is often a temperature change following the Joule-Thomson coefficients ($\mu_{JP} = \Delta T/\Delta p$), because the hydrothermal fluid must do external work against the ambient air to consume thermal energy. Using the flow reactor, we have demonstrated oligomerization of glycine up to decamer (Gly_{10}), which have never been yielded with any other quenching methods. In this paper, the efficiency of rapid quenching methods such as adiabatic expansion will be discussed in prebiotic synthesis of hydrolysable compounds including biopolymers.

Materials & Methods

Glycine and diglycine was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Distilled water was purchased from Gibco BRL and Kozakai Seiyaku Co., Ltd. As authentic samples for LC, diglycine and glycine anhydride (diketopiperazine) were purchased from Wako Pure Chemical Industries, Ltd., tri-, tetra-, penta- and hexaglycines were from Sigma, and hepta- and octaglycines were supplied as a custom synthesis from Invitrogen Co.

The flow reactor with adiabatic expansion cooling was composed of two reservoirs, pumps, a pre-heater, a 1.5 mL of flow reactor with heaters, and a needle valve as described previously [7]. Most parts of the hydrothermal reactor were made from SUS316. The orifice was formed by the needle valve and its valve seat made from nickel-based alloy, Inconel 625 (including chrome with a slight amount of molybdenum and niobium) and Hastelloy C-22 (including chrome and molybdenum with a slight amount of iron and tungsten), respectively.

High performance liquid chromatography (HPLC) analysis was performed using an HPLC apparatus, Waters 600E/2487 system attached with an octadecyl bonded silica-based column, X-Terra MS C_{18} (Waters, ϕ = 2.5 μm; 4.6 mm, inner diameter; 50 mm length). As oligopeptides consisting of glycine residues, which have no side chain, are too hydrophilic to be analyzed using reversed-phase liquid chromatography (RP-LC), we applied ion-pair RP-LC [10,11]. The mobile phase consisted of 5 mmol L^{-1} potassium dihydrogen phosphate (KH_2PO_4, Wako Pure Chemical Industries, Ltd.) and 2.5 mmol L^{-1} sodium 1-hexanesulfonate ($C_6H_{13}SO_3Na$, Sigma-Aldrich Co.), and the pH was maintained at 2.5 with phosphoric acid (H_3PO_4, Sigma-Aldrich Co.). The flow rate of the mobile phase was 0.5 mL min^{-1}. Before loading, the sample was filtrated by a 0.22 μm

centrifugal filter device (Millipore Ultrafree-MC). The UV detector monitored the absorbance at 200 nm to detect oligoglycine series.

Liquid chromatography / mass spectrometry (LC/MS) was performed using a module of Waters 2695 and Micromass Quattro micro ZQ2000. As the mobile phase for LC/MS, a volatile ion-pair reagent, undecafluorohexanoic acid ($C_5F_{11}COOH$, Tokyo Kasei Kogyo Co. Ltd.) was used at the concentration of 1 mmol L^{-1} [12]. Mass numbers were scanned with positive and negative ion mode, but no signals were detected with negative ion mode.

Results

To simulate rapid quenching from hydrothermal conditions, we designed a new hydrothermal flow reactor with adiabatic expansion cooling and performed hydrothermal oligomerization of glycine. First, the pressure of fluid was maintained at 10 MPa with a backpressure regulator. Pressurized aqueous solution of 2 mol L^{-1} of glycine was mixed with pre-heated water to be 1 mol L^{-1} of glycine aqueous solution at 270 °C. The flow rate was 2.6 mL min^{-1} and the residence time at high pressure and temperature was about 30 seconds. Then, the solution was quenched and depressurized through the orifice into the ambient air with the adiabatic expansion method. Almost all the solution was collected in spite of some evaporation. As shown in Fig. 2, LC analysis was performed and various oligoglycines were detected. Production of olygomers was confirmed by comparing with authentic samples (up to octamer) and LC/MS analysis.

LC/MS with selected ion recording mode was also performed and qualitatively detected nona-

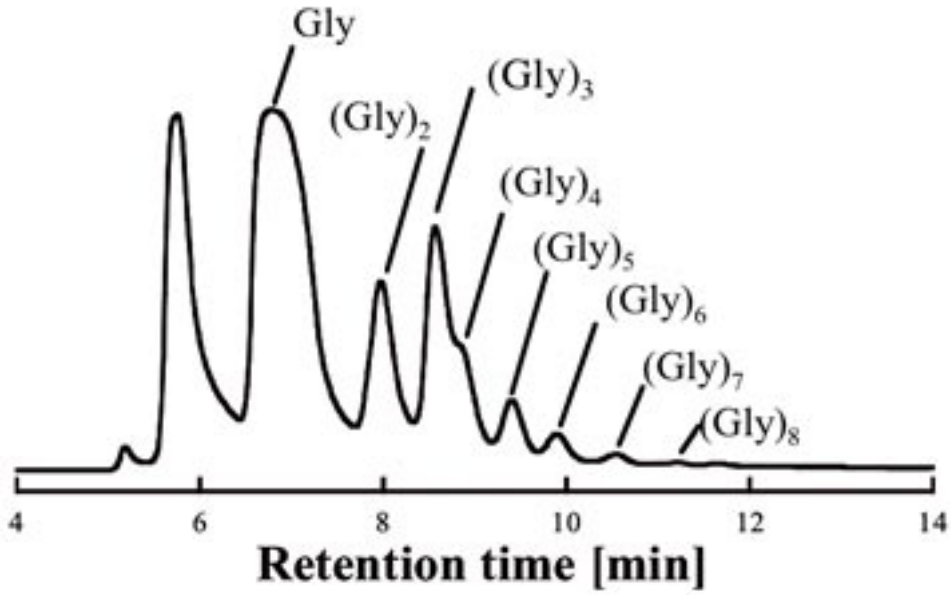

Fig. 2. Chromatogram of the products in the sample that reacted from 1 mol L^{-1} glycine aqueous solution at 270 °C and 10 MPa.

(Gly_9) and decaglycine (Gly_{10}), of which protonated mass $[M+H]^+$ is 532.2 and 589.2, respectively (data not shown), that had never been obtained with any other cooling methods.

Discussion

In this paper, we demonstrated that the adiabatic expansion cooling method was one of the most rapid quenching processes to polymerize peptides from amino acid. The qualitative analysis shows that longer peptides were synthesized than in the previous works [1-5]. This suggests the rapid quenching method is quite effective to synthesize polymer from monomer. We cannot, however, lead to the conclusion whether the rapid quenching would avoid hydrolysis of oligomers synthesized in the hydrothermal reactor, or whether the rapid quenching would facilitate dehydration condensation of glycines as a result of evaporation of solvents and nucleation of solutes.

Our hydrothermal flow reactor with adiabatic expansion cooling is simulated as hot springs, geysers, fumaroles and volcanic eruptions rather than deep-sea hydrothermal vents. Rapid quenching such as volcanoes would cause more drastic change of pressure and temperature than deep-sea vents. Concerning with efficient dehydration condensation, such a non-equilibrium condition would be a good environment for accumulation of biopolymers. Although we demonstrated only oligoglycine synthesis, the rapid quenching from hydrothermal conditions would be widely useful for hydrolysable bond formation, for example, not only peptide bond but also phosphoester, glycoside, acyl bond and so on.

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