FORMATION OF AMINO ACID CONDENSATES PARTLY HAVING PEPTIDE BONDS IN A SIMULATED SUBMARINE HYDROTHERMAL ENVIRONMENT

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Abstract

Although abiotic polymerization of amino acids is an important step in origin of life study, it has often eluded that their formation could be limited in the hydrothermal systems. To show this we heated an aqueous solution of several amino acids in a simulated hydrothermal environment to assess the possible formation of peptides among the heated products. Peptide concentrations estimated by the Lowry method were significantly higher when the mixture was heated at 300°C than those at lower temperatures, despite having more than 80% of the initial amino acids decomposed. We also revealed that the peptides measured here were only part of the bonds in the heated products. The major heat products were non-peptide amino acid condensates (NPACs) that only possess partial peptide bonds. The role of NPACs should be examined though they were often ignored in the classical chemical evolution scenario so far.

(Keywords)
hydrothermal systems, peptides, non-peptide amino acid condensates (NPACs)

Introduction

Deep-sea hydrothermal environments are an important area in the study of life's origin [1]. Due to the possible availability of starting materials such as CH_4, C_2H_6 and CO with heat, the hydrothermal environment is a plausible spot to spark the earliest life forms on earth [2]. Many experiments were conducted using simulators to form biologically relevant compounds (i.e. amino acids) [3,4] and/or to test their survivability [5].

In recent years, there have been increasing interests in the formation of polypeptides, where researchers have shown that oligomerization of glycine up to hexamers could be obtained under hydrothermal conditions in some simulators [3,4,6,7]. One limitation to these kinds of experiments is that; a high concentration of the amino acid (glycine) ranging from 100 mM - 2 M was often used to amplify the yield that are usually at trace levels [3,4,6,7]. Aubrey and co-workers [8] argues that, such high concentration is unlikely in the prebiotic ocean and their reaction threshold is often exceeded. They estimated that the carbon reservoir in early ocean are limited and constitute a small value only, for example HCN was about 2 micromolar [9] and additionally, ammonia was about 20 micromolar [10]. Both of these compounds are important starting material to generate abiotic amino acids.

It is also worth noting, that the heat products of glycine in the system are relatively simpler to predict due to its simple structure than in the case of using multiple amino acids in the system. Complication could arise simply due to reaction among the amino acids' side chains. From previous experiments [5], it is well understood that amino acids exposed to hydrothermal heat (in simulators) do recover better when they are hydrolyzed, suggesting aggregated and/or condensed product in the products [5]. One question that needs to be asked, however, is whether all these product turn into genuine peptides or not when complexity is added, particularly in this case that different kinds of amino acids are used. A number of studies suggested that aggregates or globules-like structures were formed [11,12,13], when various amino acids were heated at high temperature. For example, Yanagawa and Kojima [11] demonstrated that microsphere-like structure were generally formed when a mixture of glycine (0.3M), alanine (0.1M), aspartic acid (0.1M) and valine (0.3M), were heated in a hydrothermal simulator (autoclave) at 250°C or 300°C. Similarly, Fox and Harada [12] showed the formation of proteinoid microspheres when 18 kinds of amino acids with a higher concentration of dicarboxylic amino acids (i.e. aspartic acid and glutamic acids) were heated. These kinds of structures have been generally envisioned to play a role in chemical evolution [11,12,13,14].

This paper attempts to show the features of thermal condensates of amino acids in submarine hydrothermal simulator, the Super Critical Water Flow Reactor (SCWFR) (Figure 1) [3,5]. The survivability of the amino acids was quantitatively examined. We then characterized the products by infrared spectrometry and the Lowry assay. Although similar experiments have shown peptides of glycine in the product, no work has yet estimated the peptide yield when plural kinds of amino acids are added in the system.

Material and Method

We used a mixed solution of glycine, alanine, aspartic acid and valine at 20 mM each, being lower concentration compared to previous works [3,4,6,7,11,12], and exposed them at room temperature (20 °C), 200°C, 250°C or 300°C at 25 MPa. The sample was injected through the IR image gold furnace for a 2 min of heat exposure at a flow rate of 0.5 mL/min in the SCWFR (Figure 1) (See Islam et al, 2003 [3] for apparatus details). We decided to use these amino acids based on the GADVs peptide world [15] and Trifonov's amino acids temporal order [16].

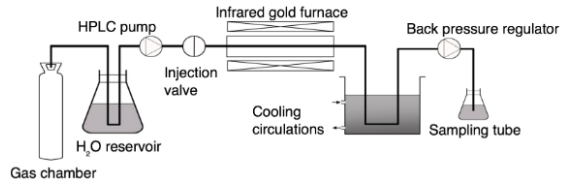

Figure 1: Schematic diagram of the Super Critical Water Flow Reactor (SCWFR)

A portion of the samples were then hydrolyzed with 6 M HCl for 24 hours at 110°C. Both hydrolyzed and non-hydrolyzed portions were desalted using a solid phase extraction spin column Monospin-SCX provided by GL Sciences and analyzed using an ion exchange HPLC, which is made up by two HPLC pumps. (Shimadzu LC-10), a cation exchange column (Shimpak ISC-07/S1504, 4 mm i.d. x 150 mm), a post-column derivatization unit, and a Shimadzu RF-535 fluorometric detector. N-acetyl-L-cysteine (N-AcCys) and o-phthalaldehyde (OPA) were used as derivatizing agents in borate buffer. Procedure blanks was done in every steps of the experiments (simulation, hydrolysis, desalting and analysis).

The products (non-hydrolyzed) at all temperature were analyzed using a Fourier transform infrared spectrometer (JASCO FT-IR-6200) to compare their structure. Freeze-dried products were pressed between two KBr plates before analysis.

An aliquot of the samples was also used to determine the occurrence of peptide bonds using the Lowry assay protocol [17,18,19]. The method was chosen because of it's sensitively to low concentration to peptides. Conventionally, bovine serum albumin (BSA) has been used as a standard in most protein calorimetric assay. In this study, however, pentaglycine was preferred instead to compensate BSA's tyrosine and tryptophan residues and -SH groups [20]. These amino acids are known to enhance the colour yield of the Lowry solution, which could underestimate the concentration of peptide bonds in the products. Absorbance was recorded using a JASCO V-630 UV-spectrophotometer at 750 nm.

Additionally, scanning electron microscopy (SEM) was utilized to access any physical traits in the heated products, after trapped on a 0.2-micrometer pore size polycarbonate membrane filter or on clean aluminum foil.

All experiments were done using Milli-Q water and analytical grade chemicals. Amino acids, BSA, pentaglycine and Lowry solution's chemicals (sodium tartrate dihydrate, $CuSO_4 \cdot 5H_2O$, NaOH, Na_2CO_3) were purchased from Wako pure chemicals. Only Folin and Ciocalteu's Phenol Reagent was obtained by Sigma-Aldrich. All glassware were baked at 500°C before using for all experiments.

Results and Discussion

Figure 2 shows the recovery of amino acids before and after hydrolysis. The present results were

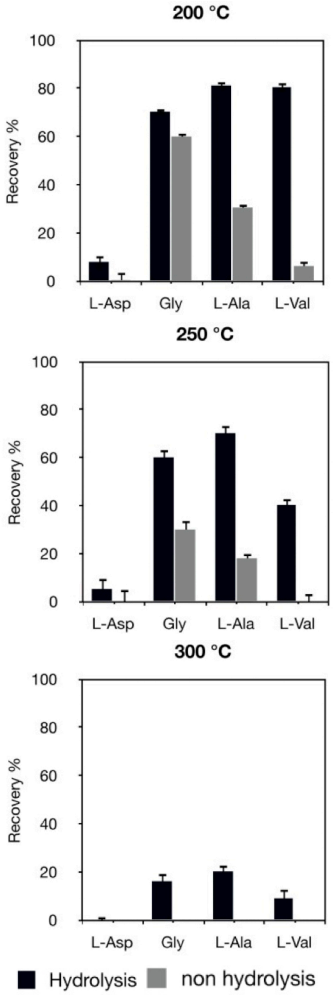

Figure 2: Recovery ratio (%) of amino acids after heated in the SCWFR at different temperature. Amino acids in each sample were determined before and after hydrolysis.

Table 1: Estimation of peptides concentration and their contribution to the total combined amino acids in the heated products.

Temperature	% of free amino acids[a]	Σ Combined amino acids after heating (g/L)[b]	Peptide Conc. (mg/L)[c]	Corrected Peptide Conc. (mg/L)[d]	%[e]
200 °C	60	3.00	0.699	-0.2256	-0.01
250 °C	30	2.46	6.842	6.3797	0.26
300 °C	0	0.81	75.39	75.39	9.26

[a] Percentage of free amino acids in the total recovered amino acids after heating

[b] Concentration difference between hydrolyzed and non hydrolyzed portions (based from Figure 1) to produce the yield of combined amino acids

[c] Peptide concentrations estimated by using pentaglycine as a standard (based on Figure 3) in the non-hydrolyzed portion

[d] Peptide bonds corrected by subtracting free amino acids' interference[a] in the non-hydrolyzed portions

[e] Percent contribution of peptide bond to the total combined amino acids based on the corrected values[d]

consistent with previous works [3,5], where the recovery ratio decreased with the increment of temperature. The difference between hydrolyzed and non-hydrolyzed portion were referred to as combined amino acids' yield (Table 1). The 300°C samples exhibit the lowest yield indicating an overall 20% or

less recovery of amino acids, with no free amino acids in their non-hydrolyzed portion (Figure 2). Additionally, glycine and alanine showed higher recovery than valine and aspartic acid; aspartic acid was almost depleted at this temperature.

The FT-IR analyses showed that the 200°C and 250°C spectra were somewhat similar to that of room temperature (control) (Figure 3), indicating the interference of coexisting free amino acids in the heated samples. The 300°C spectrum, however, was significantly different from the others. Absorbance of amide I (1600-1700 cm-1) and II (1510-1580 cm-1) regions were found in the 300°C sample (Figure 3), which might be due to the presence of peptides in the heated products.

The Lowry assay, used to quantify the peptides showed that the absorbance of the room temperature (control) sample was 0.02 (Figure 4), inferring the

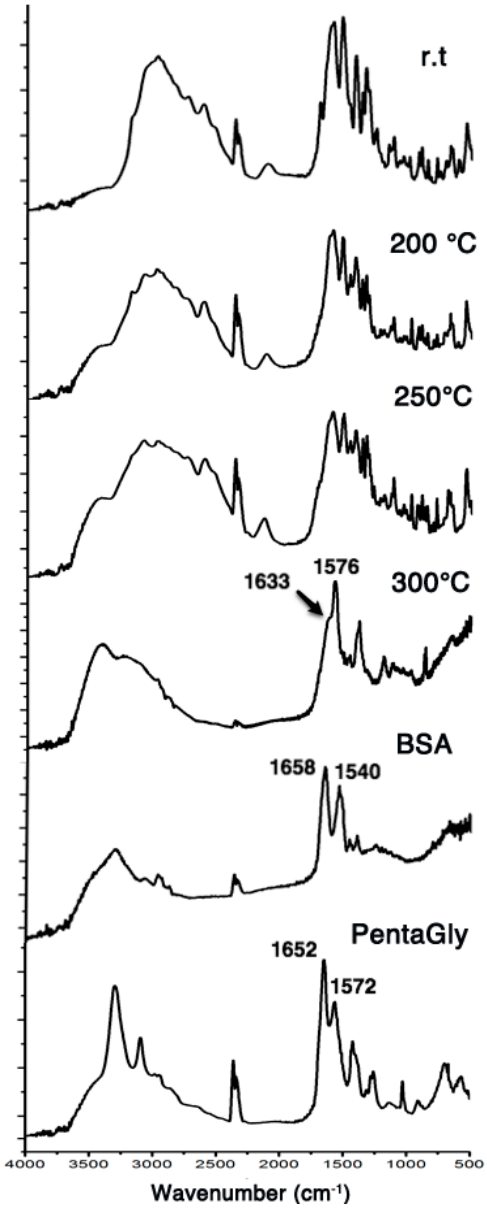

Figure 3: FT-IR absorbance spectra of the heat products. Pentaglycine and BSA are also shown as references. r.t. = room temperature.

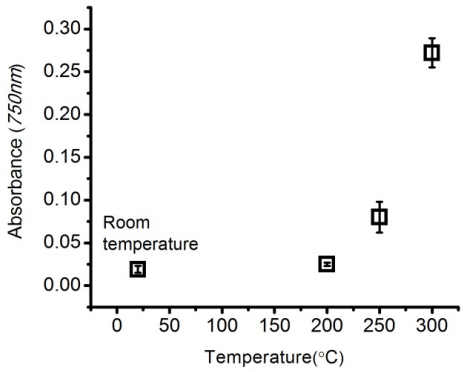

Figure 4: Absorbance of the heat products by the Lowry assay

positive interference of free amino acids in the solution [21]. The 250°C samples showed slightly higher absorbance 0.08 and the 300°C samples exhibited a very prominent absorption of about 0.27 (Figure 4). Our results suggested that the possible formation of prebiotic peptides could be more favorable in hydrothermal conditions, especially at 300°C. As mentioned above, we used pentaglycine to estimate the peptide concentration: The estimated concentrations were 0.69 mg/L (200°C), 6.84 mg/L (250°C) and 75.4 mg/L (300°C), respectively. After subtracting positive interferences by free amino acids [21] corrected concentrations of peptide bond were almost 0 mg/L (200°C), 6.4 mg/L (250°C) and 75.4 mg/L (300°C) (Table 1). These contributed about 0.3% (250°C) and 9.3% (300°C) of the total combined amino acids in the heated products at each temperature. The contribution of the peptide bonds in the amino acids condensates produced at 300°C was significantly higher than those produced at lower temperature. The majority of the products were what we would define as non-peptide condensates (NPACs) for the all heated sample. Since aspartic acids were mostly depleted (Figure 2), we predict that only glycine, alanine and valine were involved in the thermal condensates when heated at 300°C.

The present findings that NPACs were major products derived from plural amino acids in the simulation experiments suggested that such complex molecules could also be major components in the actual prebiotic setting. One would assume that using singular amino acids (*i.e.*, glycine) does not show the complete picture of oligomerization in hydrothermal environment. Compounds such as NPACs, often ignored in the classical scenario, could possess some chemical functions relevant to origins of life. Although we have not shown their functionality here, a similar experiment was done by Oba and co-workers [19], who performed a series of experiments using a heat-drying and wet cycle technique (on a mixture of glycine, alanine, aspartic acid and valine) to simulate peptide formation based on the GADV's protein world hypothesis [15]. After 30 cycles of heat-drying and wet, their products that also exhibit peptide bond occurrences (by Lowry Method) could perform hydrolytic activity in the presence of BSA solutions. Since we applied different process to simulate prebiotic amino acid

condensation from their heat-drying and wet cycles, their findings might not be directly transferable to ours. Therefore, studies on NPACs' functionality should be done in future.

It is also noteworthy that any sort of microsphere-like structures [11,12] were not detected by SEM in our heated samples (data not shown). As mentioned earlier, Yanagawa and Kojima [11] demonstrated that their microspheres were generally formed when the four amino acids were heated at 250°C or 300°C. However, the amide II band peak were not detected in their IR spectra; and the role of silica from glass tube was also emphasized to form such structures. In this study, we used the same four amino acids as their experiment but at lower concentration. We also avoided using glass tube during the heating process hence eliminating the possible influence of silica. However, despite the lack of microsphere-like structures, the 300°C sample was showing similar attributes to proteinoid microsphere [12] demonstrated by Fox and Harada, such as being Biuret positive [17] (Lowry method) and the presence of the amide I and II peak indicating peptide bonds. This study confirmed that formations of morphological structures are often associated with high concentration of starting compounds and/or the involvement of other inorganic [14] compounds like silica.

Conclusion

In this investigation, the aim was to quantify the thermal condensate in relation to peptides using multiple amino acids. The combinations of these data reveal that amino acids did not only degrade with heat, but also form condensation products as well. A part of the bonds in these products were peptides. However, the contribution of peptide bonds in the combined amino acids was less than 10%, even in the 300°C sample, which showed the highest contribution of peptides. The major constituents of the product were non-peptide amino acid condensates (NPACs) for all the heated samples. A limitation of this study is that we did not identify the products structure of NPACs and peptides; due to the fact that the products might be too enormous and yield of individual species would be too low to accurately pin point the compounds. However, we have shown that yield of peptides is rather low in comparison to the overall product. This research has thrown up many questions in need of further investigation. One of them is, whether NPACs have any function or not in chemical evolution and/or the origin of life. Complex organic compounds like NPACs were often ignored in the classical chemical evolution so far, since the importance of origin of life research is often focus in biologically relevant compounds (peptides) only. We recommend further experiments on the non-biologically relevant compounds like NPACs to examine their role in chemical evolution on earth and extraterrestrial bodies.

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