HYDROTHERMAL CIRCULATION OF SEAWATER THROUGH HOT VENTS AND CONTRIBUTION OF INTERFACE CHEMISTRY TO PREBIOTIC SYNTHESIS

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(Received 20 February, 2000; accepted in revised form 3 July, 2000)

Abstract. Synthesizing oligopeptides from glycine and alanine in a flow reactor, which stimulates constant hydrothermal circulation of seawater through hot vents on the primitive Earth, demonstrated that an exponential growth of the products is possible. The initial rapid growth of the product is a consequence of using the products formed in one cycle as the starting materials for the cycle of synthesis.

Keywords: alanine, exponential growth, glycine, hydrothermal vents, oligopeptides, prebiotic synthesis

1. Introduction

Hydrothermal vents on the sea floor of the primitive Earth have been proposed as likely locales among others for prebiotic synthesis (Corliss *et al*., 1979; Edmond *et al*., 1982; Ferris, 1992; Shock, 1996; Russell and Hall, 1997). Here solutions of organics are heated while circulated through hot environments and then quenched when discharged into cold water (4 °C). The thermal energy of the hydrothermal system drives bond formation and the products are more stable in the cold water.

The scheme of transforming products from preceding production into reactants for subsequent one could already have been available on the primitive Earth. This might have been so even before the evolutionary emergence of biochemical pathways converting part of the products constantly into the reactants for the subsequent reaction within closed reaction cycles.

We already attempted an experimental model simulating geological conditions for hydrothermal circulation of seawater through hot vents (Matsuno, 1997; Imai *et al*., 1999a). We observed an exponential growth of oligopeptides with the elapse of time at least initially when the circulating reaction solution was initially glycine dissolved in water (Imai *et al*., 1999b). In the present article, we shall further examine the experimental model of hydrothermal circulation for the reaction solution including more than one kind of amino acid, and detail the quantitative characteristic of the initial exponential growth of oligopeptides to be synthesized.

Origins of Life and Evolution of the Biosphere **30:** 527–537, 2000.

We shall concern ourselves with chemical reactions, those which are at the stationary state in the interface between the hot and cold region. More specifically, we shall focus on the reactions proceeding at the interface, in which oligomeric formation at the higher temperature would eventually come to equilibrate with its decomposition at the lower.

2. Simulating Hydrothermal Circulation

A principal feature of the flow reactor is, that a high-temperature, high-pressure fluid is injected into a low-temperature chamber maintaining the same high pressure, while the whole fluid is circulated in a closed manner in the system with a fixed turnover rate (see Matsuno (1997) and Imai *et al*. (1999a) for details). The high-temperature, high-pressure fluid was prepared in a pressurized and heated section of the closed circuit of the fluid, whereas the low-temperature chamber was maintained by immersing it in an external cooling apparatus. The high-temperature, high-pressure fluid jet from a nozzle into the low-temperature chamber was intended to simulate a submarine hydrothermal vent. At the same time, the fluid flowing out of the low-temperature chamber was first depressurized to normal atmospheric pressure to sample a very small fluid volume for the purpose of measurement at a given time interval and then followed by conversion back to the high-temperature, high-pressure fluid.

The reactor was designed to circulate the fluid in a unidirectional manner; from the high-temperature, high-pressure chamber through a nozzle into the low-temperature, high-pressure chamber connected further downstream to a long needle tube for depressurization to normal atmospheric pressure for sampling access, then through a pump for repressurization to the high-temperature, high-pressure chamber through a tube. All the materials for the chambers and tubing were made of stainless steel *SUS316*, to prevent corrosion. However we observed a slight color change of the inner wall of the high temperature chamber into a more blackish one after continuous operation over 50 hr at the pH 2.5 and at temperature 200–250 °C. But, ferrous ions over 0.1 ppm were not identified when the reaction solution in our experiments was subject to bipyridine-treated colorimetric analysis at 522 nm.

Heating of the high-temperature, high-pressure chamber was furnished by a nichrome-wire electric heater attached to its outer surface, and the temperature was monitored by a thermocouple. The volume of that chamber was 15 mL. The tube connecting the high-temperature, high-pressure chamber to the low-temperature, high-pressure chamber was 50 mm long with a diameter of 800 μm. The 250 mm-long low-temperature, high-pressure chamber with a diameter of 20 mm and a volume of 78.5 mL was immersed in a 20 L water bath contacting a cooling pipe carrying coolant at –20 °C. The water in the bath was constantly alternated, and the cooling system was controlled so as to maintain the temperature of the outer surface of the chamber at the downstream end at 0 °C. The fluid flowing out of the low-

temperature, high-pressure chamber was connected to a 0.5 m-long capillary tube with a diameter of 100 μm, through which the fluid loses its pressure to normal atmospheric pressure. Sampling was accomplished there at fixed time intervals. The total volume of the reaction solution was 500 mL.

3. Results

We first prepared 500 mL solution of 40 mM L-alanine and 10 mM glycine at pH 2.5 adjusted by HCl at room temperature. The pressure of the high-temperature, high-pressure chamber with a volume 15 mL was set at 24.0 MPa, which is only slightly above the pressure of the critical point of water (22.1 MPa). The flow rate was about 10 mL min^{-1}. This gave the cycle time 34 sec of reactants rounding the closed flow circuit in stirred conditions (Imai *et al*., 1999b). The temperature of the high-temperature, high-pressure chamber was set at 250 °C. The time required for reaching the designated temperature from room temperature took about 10 min. Figure 1 demonstrates a high performance liquid chromatography (HPLC) profile of the products after 1 hr operation of the flow reactor. We have identified at least six different oligpeptides; Ala-Gly, Gly-Ala, Ala-Ala, Gly-Ala-Ala, Ala-Ala-Ala, Ala-Ala-Ala-Ala, where Ala stands for L-alanine and Gly for glycine. The retention time of each peak identified was confirmed by comparing with standards prepared independently. Time courses of the yields are demonstrated in Figure 2. The initial exponential growth rates for Ala-Ala, Ala-Gly and Ala-Ala-Ala were found almost similar, while the rates for Gly-Ala-Ala and Ala-Ala-Ala-Ala were much less. The yields of the dimers, though diketopiperazine excluded, from the initial monomers were about 3% at 10 min after the start of the operation.

We have also examined the similar reactions at pH 9.5 adjusted by NaOH and at pH 6.0 with neither acidic nor alkaline adjustment at room temperature, with other conditions being equal to the case of pH 2.5. The products of the same species as at pH 2.5 were identified. The initial exponential growth of the products was observed also at pH 6.0 and 9.5.

In order to estimate the contribution of glycine to oligomerization of alanine, we did the similar measurement for the reaction solution of 40 mM L-alanine at pH 2.5 adjusted by HCl at room temperature, while other conditions remained the same. The result on the time courses of the yields is shown in Figure 3. The initial exponential growth rates differed among the productions of Ala-Ala, Ala-Ala-Ala and Ala-Ala-Ala-Ala.

One factor that influenced the initial exponential growth of the products is the flow rate of the reaction solution. For this purpose, we tried the reaction solution of 100 mM glycine at the pH 2.5 adjusted by HCl at room temperature for two different flow rates. One was set 10 mL min^{-1}, and the other was set 4.5 mL min^{-1} by replacing the depressurization capillary tube by a longer one of 1 m length. Other conditions remained the same as previously. Time courses of the yields are

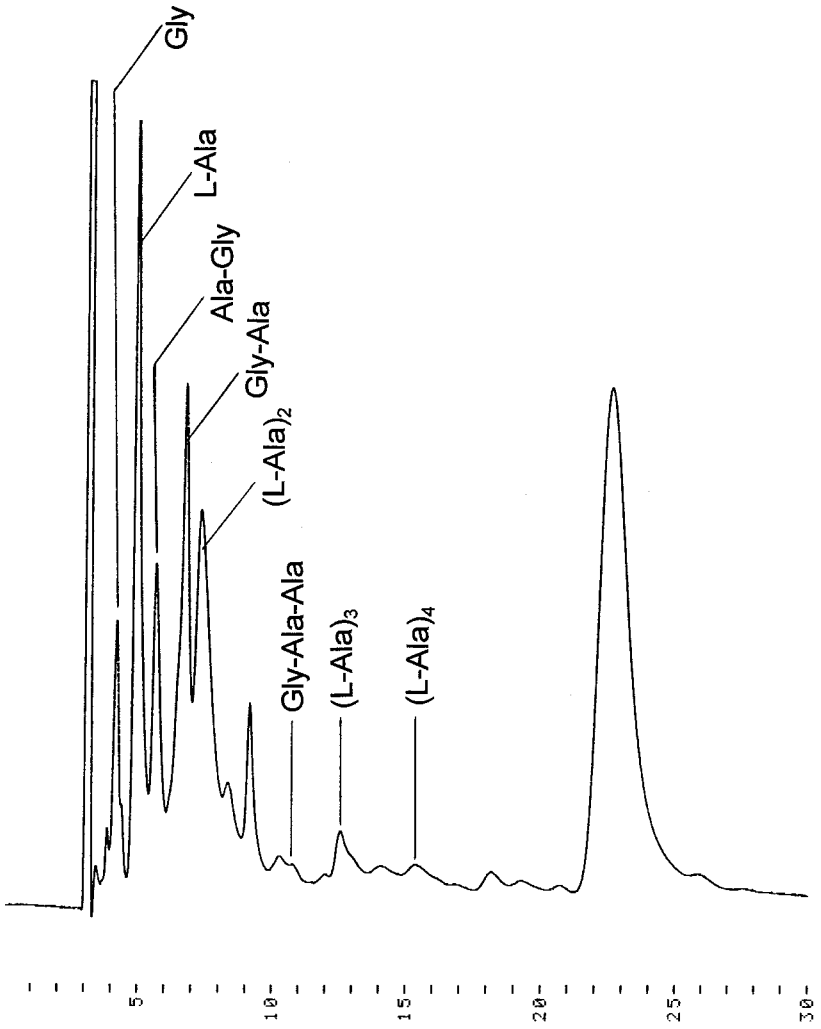

Figure 1. An HPLC profile of the products after one hour operation. All samples were analyzed by a Hitachi (*L-6300*, *L-4200* and *D-2500*) HPLC apparatus with the use of a Shodex Asahipak (*ODP-50* 5 μm/4.6 $\times$ 150 mm) column. The mobile phase consisted of 50 mM KH_2PO_4 and 7.2 mM $C_6H_{13}SO_3Na$, and its pH was maintained at 2.5 by adjusting the amount of H_3PO_4. The flow rate of the mobile phase was 0.5 mL min^{-1}. Detection was done by measuring the absorbance at 195 nm. All the identified fractions were confirmed by comparing with the standards, which were prepared independently.

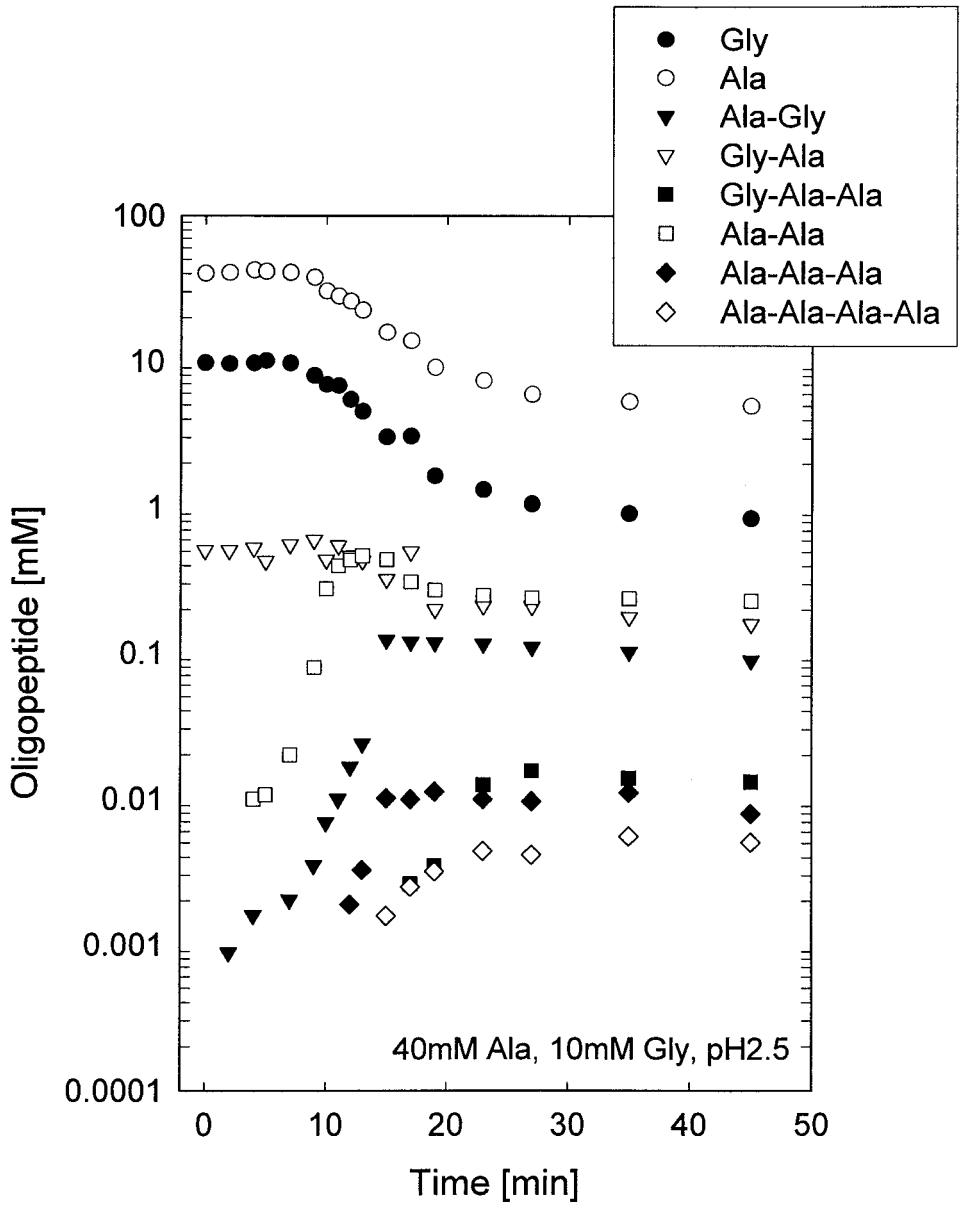

Figure 2. Time courses of the yields of the oligomers from the reaction mixture of 40 mM L-alanine and 10 mM glycine at pH 2.5 adjusted by HCl at room temperature. The origin of the time coordinate was taken to be the time point, when the temperature of the high-temperature, high-pressure chamber reached the designated 250 °C. The abbreviations are Ala for L-alanine, and Gly for glycine.

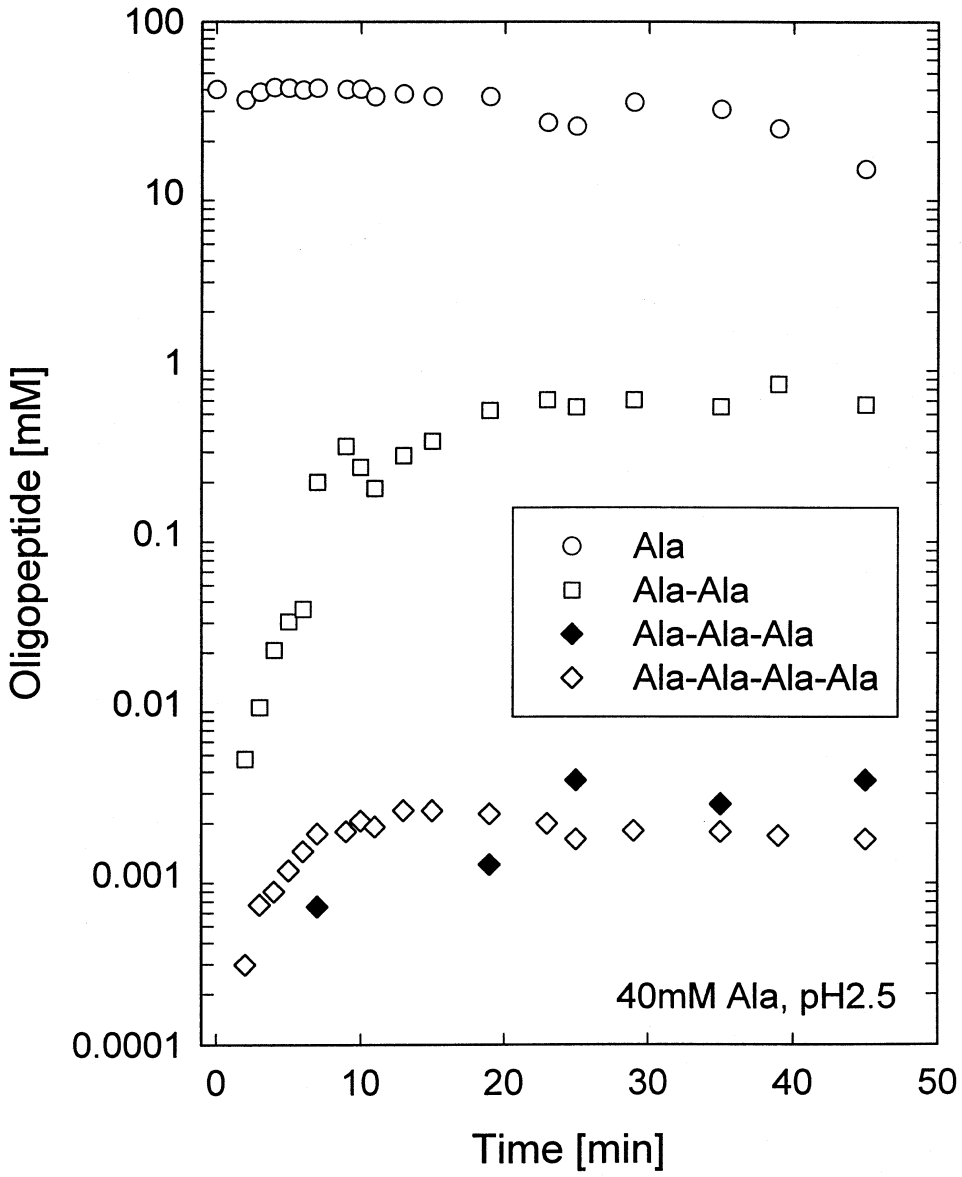

Figure 3. Time courses of the yields of the oligomers from the reaction solution of 40 mM L-alanine at pH 2.5 adjusted by HCl at room temperature. The origin of the time coordinate was taken to be the time point, when the temperature of the high-temperature, high-pressure chamber reached the designated 250 °C. The abbreviation is Ala for L-alanine.

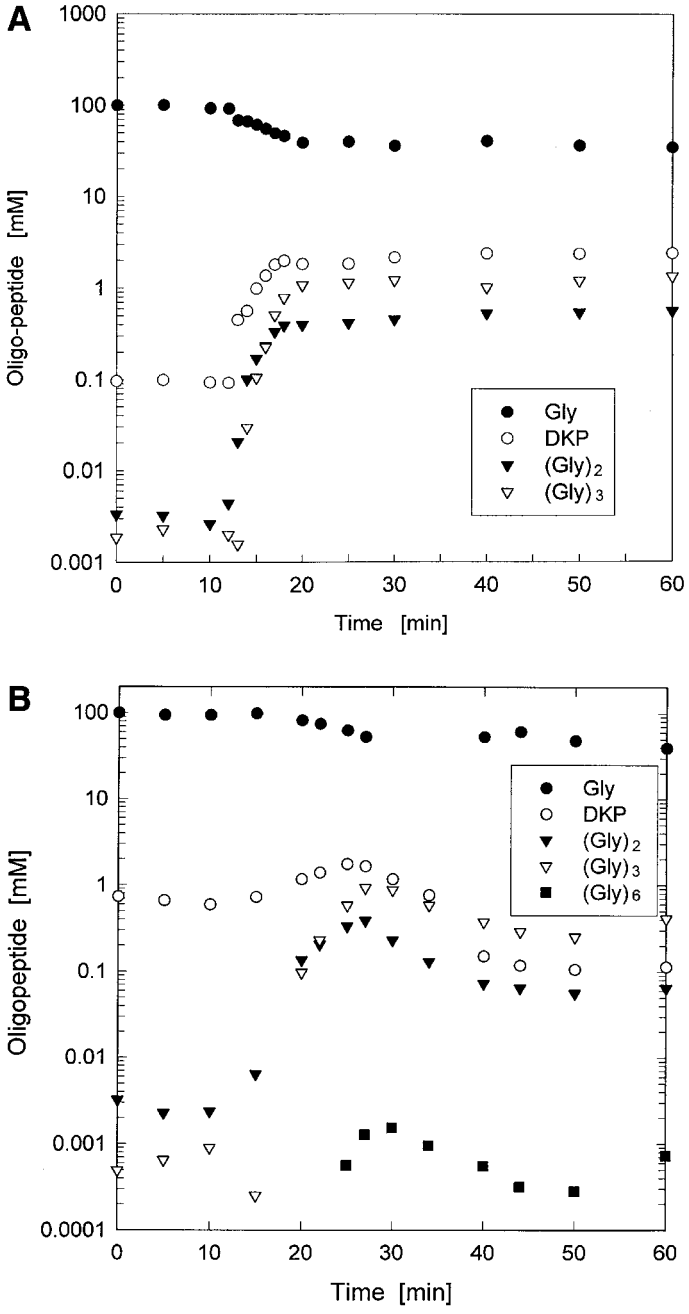

Figure 4. Time courses of the yields of the oligomers from the reaction solution of 100 mM glycine at pH 2.5 adjusted by HCl at room temperature. The flow rate of the fluid was (a) 10 mL min^{-1}, and (b) 4.5 mL min^{-1}. The origin of the time coordinate was taken to be the time point, when the temperature of the high-temperature, high pressure chamber reached the designated 250 °C. Gly for glycine, DKP for diketopiperazine, $(Gly)_2$ for diglycine, $(Gly)_3$ for triglycine, and $(Gly)_6$ for hexaglycine.

shown in Figure 4. As the flow rate decreased, the initial exponential growth rates of both diglycine and triglycine certainly decreased (c.f. Figures 4a and b).

One more factor influencing the initial exponential growth is the starting conditions. This aspect could be more clearly identified if the time course of the yield is observed for a longer period or if the initial reaction conditions are selectively chosen. For this purpose, we examined the reaction solution of 100 mM glycine and 10 mM $CuCl_2$ as a source of metallic catalysts at the pH 2.5 adjusted by HCl at room temperature and measured the reaction products for 2 hr. Other conditions remained the same, including the depressurization capillary tube of 0.5 m length. The results are shown in Figure 5a. Synthesis of peptides up to octaglycine was identified. For comparison, we also examined the case of the previous reaction solution with added 10 mM diketopiperazine, to see the contribution of the initial reaction conditions. The results are presented in Figure 5b. What significant was in this change of initial conditions, was enhancement of the initial exponential growth of di- and tetraglycine.

4. Discussion

Initial exponential growth of oligopeptides in the flow reactor simulating hydrothermal circulation of seawater through hot vents on the primitive earth, demonstrated that part of the earlier products from the previous cycle serves as reactants for the succeeding cycle, with the consequence of enhancing the synthesis of the products of like kinds exponentially in time at least initially. Repeated cycles of elongation and splicing of oligopeptides can certainly underlie such an exponential growth, in which the major factor of the splicing is hydrolysis of oligopeptides synthesized in hot regions. Of course, there should be a legitimate reminder that for those oligomers, whose residence time inside the hot vents was too large, the dissociation or degradation processes inside the vents would definitely be overwhelming (Bada *et al*., 1995). In particular, our relatively low yield of the reaction products was due to the tradeoff between the interface chemistry for elongation and the volume chemistry arising in the repeated visiting to the hot region in which the products would eventually be vulnerable to decomposition.

The difference in the amounts of oligopeptides between Ala-Ala and Ala-Gly (Figure 2), while maintaining the similar exponential growth rate, may be due to the difference in the strengths of reaction making dipeptide Ala-Ala and Ala-Gly. The similar exponential growth rate for both the syntheses of Ala-Ala and Ala-Gly implies that peptide-bond elongation and splicing would take place in a similar manner as reactants pass round the closed flow circuit of the reactor. In contrast, the difference in the exponential growth rates between Ala-Ala-Ala and Ala-Ala-Ala-Ala seems to point to the fact that the time required for doubling the amount of tetraalanine synthesized is greater than the doubling time required for the synthesizing of trialanine. This is equivalent to the observation that the number

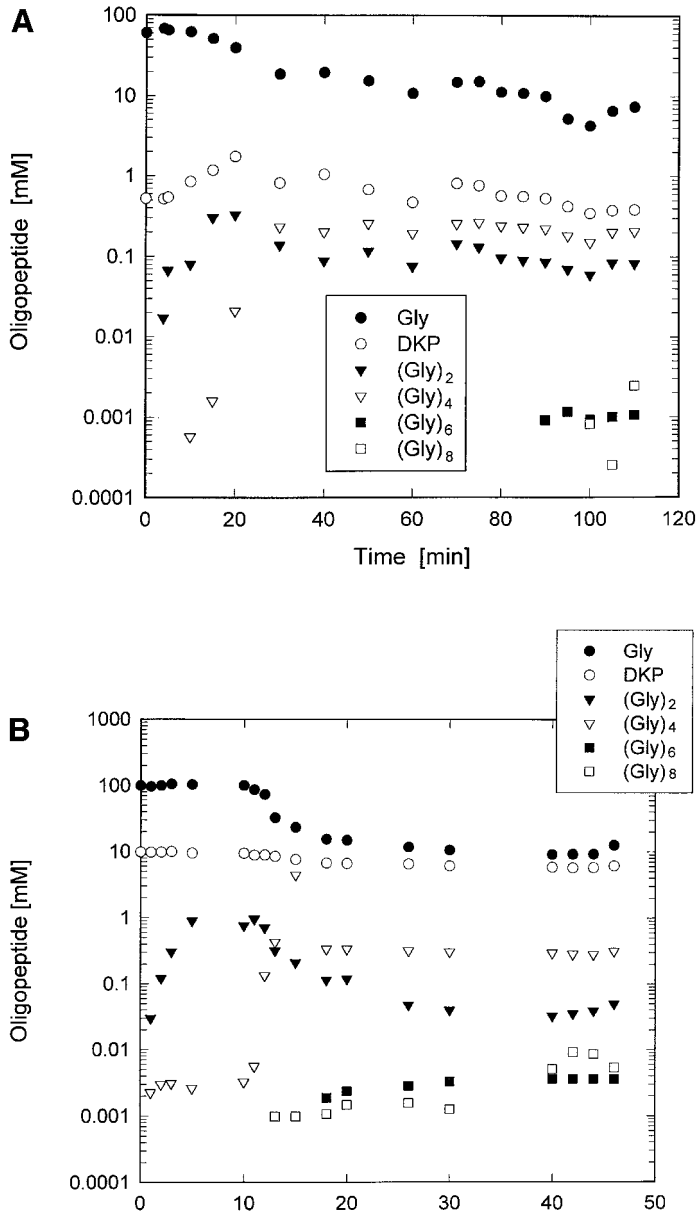

Figure 5. Time courses of the yields of the oligomers from the reaction solution of 100 mM glycine and 10 mM $CuCl_2$ at pH 2.5 adjusted by HCl at room temperature for (a), and further addition of 10 mM diketopizerazine to the initial solution for (b). The origin of time coordinate was taken to be the time point when the temperature of the high-temperature, high-pressure chamber reached the designated 250 °C. Gly for glycine, DKP for dikeropiperazine, $(Gly)_2$ for diglycine, $(Gly)_4$ for tetraglycine, $(Gly)_6$ for hexaglycine, and $(Gly)_8$ for octaglycine. Identification of the oligomers in the products was accomplished by two independent methods. One was the comparison with the HPLC elution profiles of standards up to octaglycine, and the other was due to LC mass spectroscopy up to triglycine.

of rounding the closed reaction circuit for rendering part of the products in the downstream to be identical to some reactants in the upstream is greater for the synthesis of tetraalanine. In particular, the rises of Ala-Gly, Ala-Ala-Ala, Gly-Ala-Ala and Ala-Ala-Ala-Ala coinciding with the decrease of Gly-Ala suggest that the activity of dissecting the Gly-Ala bond could enhance the production of the four oligomers.

The relationship between the initial exponential growth of oligopeptide synthesis and the cycle time required for reactants, presented in Figure 4, confirms that the time required for doubling the amount of oligopeptides synthesized increases as the cycle time of reactants increases. One of the major factors determining the exponential growth rate was found to be the macroscopic parameter specifying the flow rate of hydrothermal circulation of reaction solution. Even hexaglycine was synthesized in the absence of metallic ions when the cycle time was increased.

One more factor specifying the initial exponential growth of oligopeptide synthesis is characterized by how the initial conditions were prepared. When diketopiperazine as an intermediary product was initially added to the reaction solution of glycine, the initial exponential growth of tetraglycine was highly enhanced compared to the case otherwise as presented in Figure 5. This suggests that the average number of peptide bonds synthesized during one cycle time could be enhanced if the initial conditions are properly chosen.

5. Concluding Remarks

One significant aspect of hydrothermal vents in prebiotic evolution is the likelihood of exothermic reactions such as those making amino acids from the smaller constituent molecules there (Shock, 1996). This formative reaction is counterbalanced by the disintegrative one also available inside hot vents (Bada *et al*., 1995). We have observed one more functional role associated with hydrothermal circulation of seawater through hot vents. That is the reaction kinetics crossing over the interface between hot and cold water. The interface chemistry is slightly tilted toward formative reaction in that products made in hot vents could remain stable once they are rapidly transferred into cold seawater. Chemical reactions of evolutionary significance may have taken advantage of the interface chemistry as repeatedly visiting hot vents, especially their peripheries with cold seawater.

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