SYNTHESIZING OLIGOMERS FROM MONOMERIC NUCLEOTIDES IN SIMULATED HYDROTHERMAL ENVIRONMENTS

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Abstract. Dimers and trimers of adenosine monophosphate (AMP) were synthesized from AMP in environments simulating hot vents on the sea floor of the primitive Earth. The simulated environments were made in the flow reactor, in which an aqueous solution of reactants was circulated from the hot to the cold region repeatedly. The oligomerization proceeded most significantly when the hot reaction solution at about 110 °C was abruptly ejected into the cold environment maintained at about 0 °C.

Keywords: adenosine monophosphate (AMP), hydrothermal vents, nucleotide. oligonucleotides, prebiotic synthesis

1. Introduction

A major step in prebiotic synthesis was the formation of oligopeptides from amino acids and oligonucleotides from mononucleotides. Oligonucleotides provided both genetic information as well as catalytic function for the first life. Although the prebiotic synthesis of nucleotides from nitrogen bases, ribose and phosphate is yet to be demonstrated, piecemeal experiments have already presented somewhat positive evidence for the likelihood (Lohrmann and Orgel, 1971; Yamagata *et al*., 1991; Schwartz and DeGraaf, 1993; Pitsch *et al*., 1995; Shapiro, 1999). The next major step must have been the synthesis of oligonucleotides from nucleotides (Sleeper *et al*., 1978; Sievers and von Kiedrowski, 1994).

Mineral surfaces on the primitive earth must have functioned as major templates for the oligomerization of nucleotides if both replenishing the templates with the activated resources for the synthetic reactions and detaching the products from the templates could properly be coordinated by some means (Ferris, 1993; Kawamura and Ferris, 1994; Ertem and Ferris, 1996). A key factor here was how monomeric nucleotides were activated.

Hydrothermal environments on the sea floor on the primitive earth might also have served as locales for making oligonucleotides from nucleotides because of the presence of the natural means for both supplying the resources activated by thermal energy and quenching the products in the cold surrounding (Matsuno, 2000). The presence of constant temperature gradient between the hot vents and the surround-

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ing cold seawater could in fact have provided such a selective environment that only those products that may coexist with the gradient could survive (Matsuno, 1997; Imai *et al*., 1999a). We examined this likelihood of making oligonucleotides from nucleotides in an experimental environment simulating the constant circulation of seawater through the hot vents on the sea floor.

Chemical reactions of interest are those which are taking place at the interface between the hot and the cold region, though the equilibrium reactions moving from the hot to the cold region and back are definitely proceeding there. If the transfer of the reaction products across the interface takes less time than the time required for their decomposition at the high temperature, the products out of the equilibrium conditions could have survived longer at the lower temperatures in the region of quenching.

2. Simulating Hydrothermal Environments

We used the flow reactor already constructed for synthesizing oligopeptides from amino acids (Matsuno, 1997; Imai *et al*., 1999a) for examining the likelihood of synthesizing oligonucleotides from nucleotides. In short, a high-temperature high-pressure fluid was injected into a low-temperature chamber that was maintained at about the same high-pressure as the fluid. The fluid circulated in a closed manner in the system with a fixed turnover rate. Samples of the fluid were repeatedly taken from the low-temperature chamber for measurement at a given time interval. The total volume of the reaction solution was 500 mL, with the high-temperature, high-pressure chamber of volume 15 mL and the low-temperature, high-pressure chamber of 78.5 mL. The diameter of the nozzle connecting the two chambers was 800 μm.

3. Results

We first prepared a solution of 20 mM adenosine monophosphate (AMP) and 1 mM $ZnCl_2$ in water. The solution was adjusted to a pH of 3.0 by adding pyrophoshoric acid. Zinc ions were used because they served as catalysts for inter-nucleotide bond synthesis (Sawai and Orgel, 1975; Sawai, 1976; Lohrmann *et al*., 1980). Copper ions catalyzed peptide bond synthesis in a similar flow reactor (Imai *et al*., 1999b). Then we identified the products appeared in the flow reactor, while maintaining the high-temperature, high-pressure chamber at 110 °C and the low-temperature, high-pressure chamber at 0 °C. The pressure of the high-pressure chambers was set at 13 MPa. The flow rate of the fluid was 4.6 mL min^{-1}. An HPLC separation of the products identified 1 hr after starting the operation of the flow reactor is demonstrated in Figure 1. We used anion-exchange HPLC column Shodex IEC DEDA-420N. We eluted with a linear salt gradient while pump A delivered 20 mM

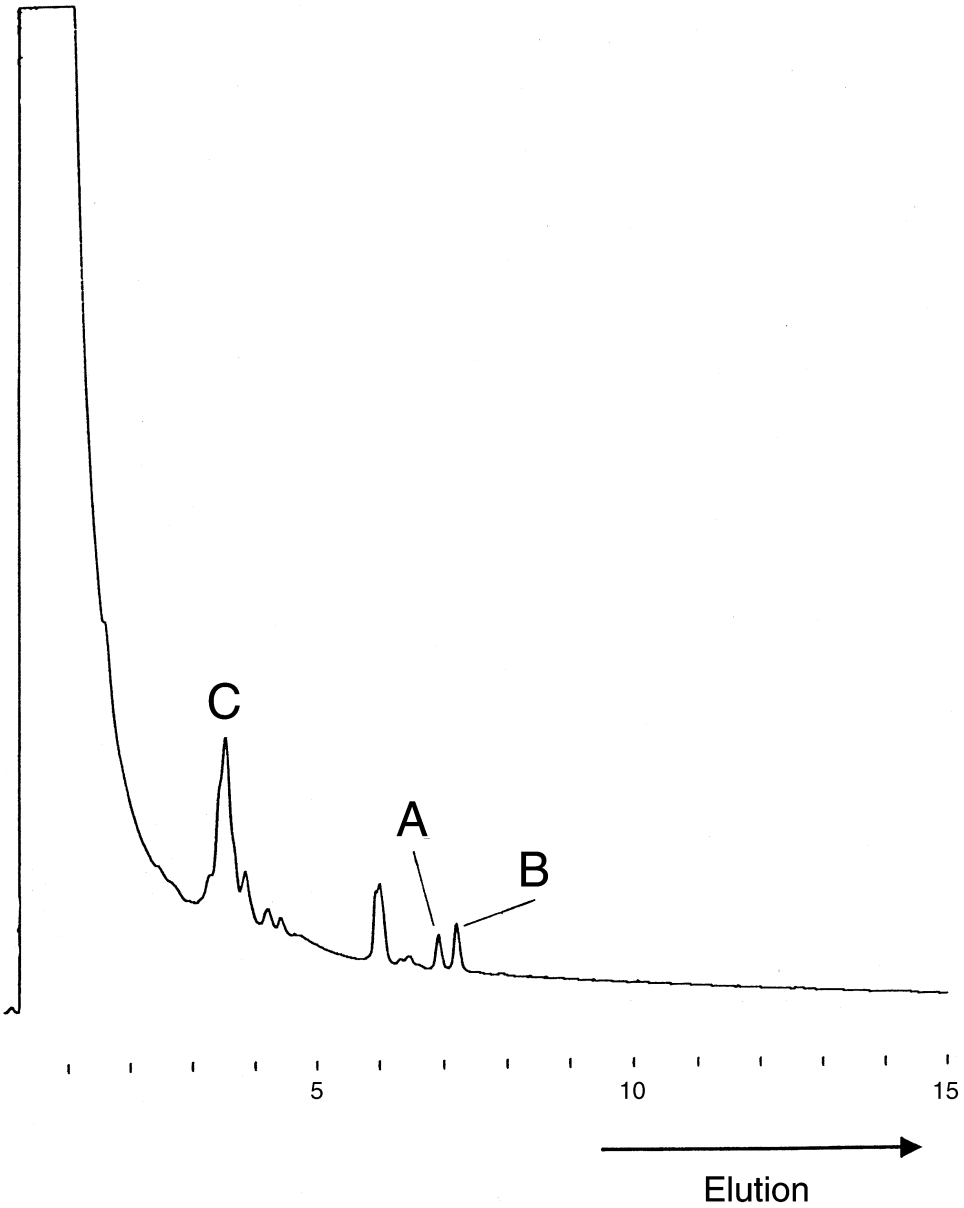

Figure 1. Anion-exchange HPLC profile of the products formed in the flow reactor 1 hr after starting the operation for the reaction solution of 20 mM AMP, 1 mM $ZnCl_2$ (pH = 3.0) adjusted by pyrophosphoric acid at room temperature, using the column Shodex IEC DEDA-420N. We eluted with a linear salt gradient while pump A delivered 20 mM Tris-$HClO_4$ adjusted to a pH of 9.0, and pump B 20 mM Tris-$HClO_4$ and 20 mM $NaClO_4$. The linear gradient went from 0.0 to 20 mM of $NaClO_4$ in 30 min at the flow rate of 1.0 mL min^{-1}. Absorbance was measured at 260 nm. The fraction marked by *A* corresponds to the trimer of adenylic acid of the 2′,5′-linkage, *B* to the trimer of the 3′,5′-linkage, and *C* to the dimer. The present identification was accomplished as referring to standards. Separation of the 2′,5′-linkage from the 3′,5′-linkage of the dimers was not feasible because of the interruption of the huge fraction due to adenosine. Separation between the 2′,5′- and 3′,5′-linkages of the trimers was additionally attempted by treatment of RNAase enzymes and was confirmed.

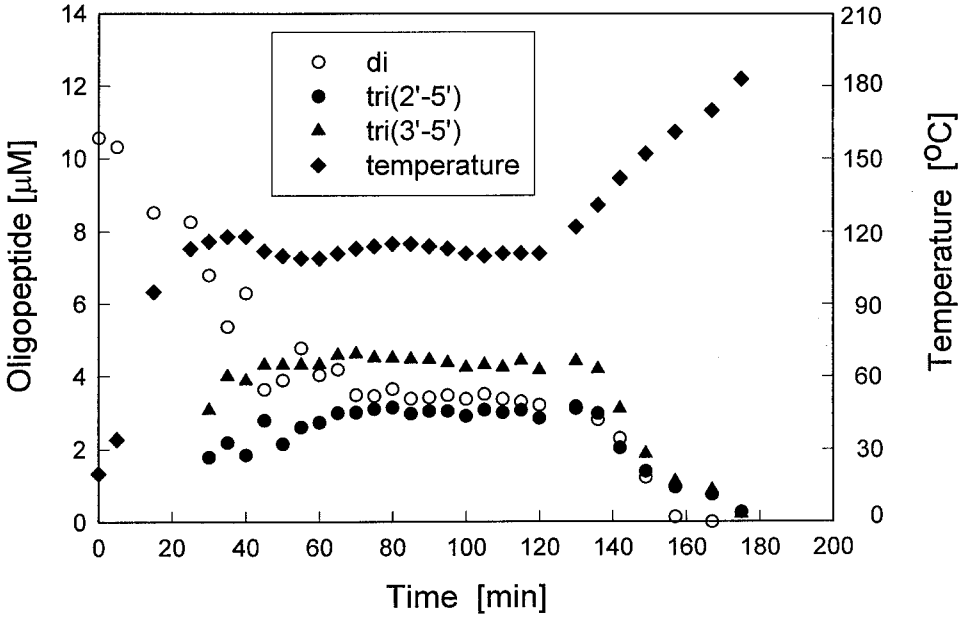

Figure 2. Time courses of the yields of the dimers and trimers in the solution conditions of 20 mM AMP, 1 mM $ZnCl_2$ at the pH 3.0 adjusted by pyrophosphoric acid at room temperature. The temperature of the high-temperature, high-pressure chamber was set at 110 °C for the first 2 hr from the start and was raised at 1 °C min^{-1} since then as presented.

Tris-$HClO_4$ adjusted to a pH of 9.0, and pump B 20 mM Tris-$HClO_4$ and 20 mM $NaClO_4$. The linear gradient went from 0.0 to 20 mM of $NaClO_4$ in 30 min at the flow rate of 1.0 mL min^{-1}. Absorbance was measured at 260 nm. The fractions *A* and *B* corresponded to the trimers, and *C* to the dimers. These fractions were identified by comparison with standards. In particular, the fraction *A* was assigned to be the trimer of the 2′,5′-linkage, and *B* to be that of the 3′,5′-linkage. Fraction *A* disappeared after treating it with venom phosphodiesterase I type 2, and *B* disappeared after treating it with nuclease P_1.

Time courses of the yields of the dimers and trimers for the reaction solution are presented in Figure 2. The high-temperature, high-pressure chamber was maintained at 110 °C for first 2 hr and its temperature was raised at the rate of 1 °C min^{-1} since then, as demonstrated there. The pressure was maintained at 13 MPa. The actual yields of the dimers and trimers from AMP obtained after 80 min of operation were 0.2%. The trimers were found to decrease as the temperature of the high-temperature, high-pressure chamber was increased above 110 °C. Although the dimers were observed even right after starting the experiments, these seem to be due to the equilibrium reaction kinetics at pressure 13 MPa. When the pressure was decreased to normal atmospheric pressure, the yield of the dimers was suppressed.

The similar result for the case of 0.5 mM zinc ions is presented in Figure 3. The observed enhancement of the dimers at about 70 min may reflect the interface

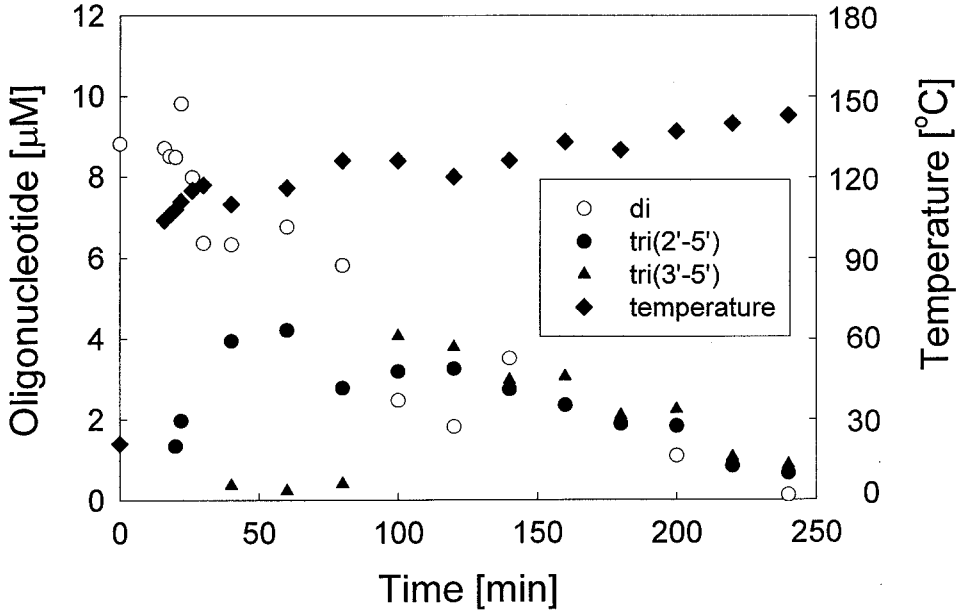

Figure 3. Time courses of the yields of the dimers and trimers in the solution conditions of 20 mM AMP, 0.5 mM $ZnCl_2$ at a pH of 3.0, adjusted by pyrophosphoric acid at room temperature. The temperature of the high-temperature, high-pressure chamber was maintained and varied as presented.

chemistry of oligomeric formation at the higher temperature and its decomposition at the lower. One reason for this delay might be that the interface chemistry proceeds in an extremely limited region spatially.

In order to see the contribution of the metallic ion catalysts more closely, the two cases of lead ions alone and of a mixture of both zinc and lead ions were further examined, as demonstrated in Figure 4. All of these results point to the fact that the best yields of triadenylate of the 3′,5′-linkage were obtained in the flow reactor when the temperature of the high-temperature, high-pressure chamber was about at 110 °C.

4. Discussion

Oligomerization of nucleotides requires energy to activate the participating monomeric nucleotides. Our result of synthesizing oligonucleotides from nucleotides, even in the absence of effective condensing agents such as the 5′-phosphorimidazolides of nucleotides, demonstrates that the rapid transfer of reactants cross the interface between the hot and cold environments does both, thermal activation of reactants and selective retention of products. Hydrothermal circulation of reaction solution through hot vents in fact provides an intrinsically selective environment such that it can thermally activate monomers inside the vents, but prevent the

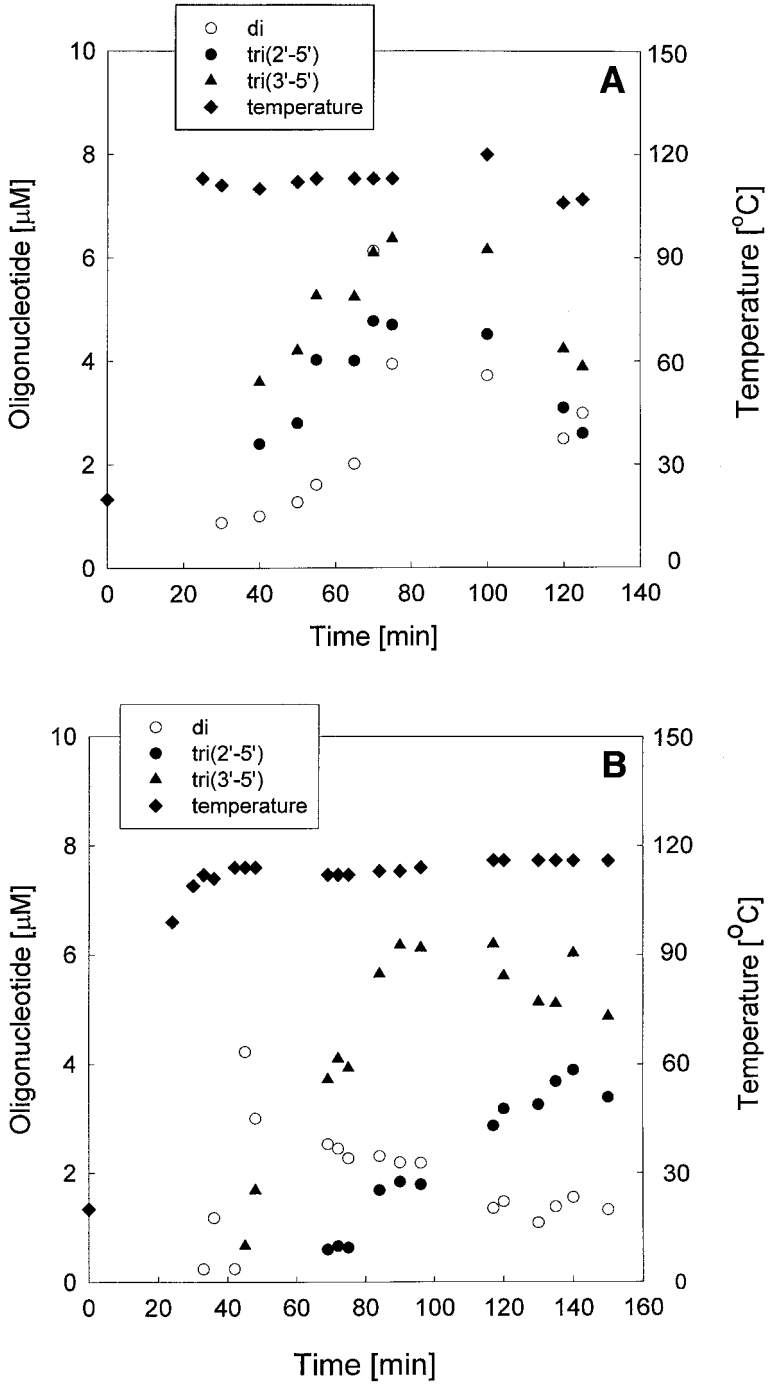

Figure 4. Time courses of the yields of the dimers and trimers in the solution conditions of 20 mM AMP, 0.5 mM $PbCl_2$ for (A), 0.7 mM $ZnCl_2$ and 0.3 mM $PbCl_2$ for (B) at a pH of 2.0, adjusted by pyrophosphoric acid at room temperature. The temperatures of the high-temperature, high-pressure chamber were maintained and varied as presented.

products from approaching the thermal equilibrium set at the temperature inside the hot vents once they leave the vents.

We observed that the most effective high temperature for synthesizing oligomers of adenosine monophosphate was around 110 °C in the hydrothermal environments. This observation suggests that if heated water is the sole means for activating monomeric nucleotides for their oligomerization, the most appropriate temperature of the water would be around 110 °C. There is evidence that archaea *Pyrolobus*, living near a hydrothermal black smoker, make their RNA and DNA at 113 °C (Blöchl *et al*., 1997). It is not known whether the last common ancestor of extant life forms was thermophilic (Galtier *et al*., 1999; Arrhenius *et al*., 1999). Rather, what concerns us at this point is whether both oligonucleotides and oligopeptides can be synthesized from their constituent monomers in a coordinated manner in the hydrothermal environments on the sea floor. One of the remaining issues from the perspective of prebiotic evolution must be if these two schemes of oligomerization could have occurred at the same time to form polypeptides and polynucletides.

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