Elongation of Oligopeptides in a Simulated Submarine Hydrothermal System

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Oligomerization of a peptide was attempted in a flow reactor that simulated a submarine hydrothermal system. When fluid containing glycine repeatedly circulated through the hot and cold regions in the reactor, oligopeptides were made from glycine. When divalent ions (such as copper ions) were added under acidic conditions, oligoglycine was elongated up to hexaglycine. This observation suggests that prebiotic monomers could have oligomerized in the vicinity of submarine hydrothermal vents on primitive Earth.

The onset of polymerization must have been a major step in the chemical evolution that formed the precursors of life (1–4). The underlying chemical reaction requires an organization in which products can be repeatedly transformed into reactants, as seen with ribosomes and ribozymes in contemporary biological organisms. Systems or processes that could have assisted the transformation of products to reactants might include heating in dry and wet conditions, the diurnal cycle, tidal waves, and dry-wet cycles in lagoons (5). Submarine hydrothermal vents (6) have been recognized as a possible environment for prebiotic synthesis; in this environment, products that were synthesized in hot vents could reenter the vents after being quenched in the surrounding cold water.

The thermal synthesis of products in hot vents (7) and the subsequent rapid cooling in surrounding cold water are generative and selective when combined (8). Thus, hydrothermal vents in the sea could have been an environment where oligomers and polymers were synthesized and selected. For instance, when two amino acid molecules form a peptide bond in hot vents and then the product is ejected into the surrounding cold water, the peptide bond could survive in the cold environment if the dissociation process [including decarboxylation, deamination, or dehydration (9)] is retarded.

We constructed a flow reactor that simulated the pressure and temperature conditions of the hydrothermal circulation of water in order to examine the likelihood of synthesizing oligopeptides from monomeric amino acids (10). However, there were still some large differences, for instance, in pH, CO₂, Na, and CI contents. In our flow reactor (Fig. 1), 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. The fluid was heated and compressed in one part of the circuit; the rest of the chamber was cooled externally. Samples of the fluid were repeatedly taken from the low-temperature chamber for measurement at a given time interval, and the fluid in the low-temperature chamber was then returned into the high-temperature high-pressure fluid.

We prepared 100 mM glycine solution that was dissolved in pure water, and we maintained the total volume of the circulating fluid at 500 mL. The pressure of the high-pressure high-temperature chamber with its 15-mL volume was set at 24.0 MPa, which is only slightly higher than the pressure of the critical point of water (22.1 MPa). This pressure was chosen to maintain the water in the chamber as a liquid.

The temperature of the high-temperature chamber was varied from 110° to 350°C in different runs. The results of interest were obtained for temperatures ranging roughly between 200° and 250°C. Temperature was increased gradually over 20 min. We started the measurements of the yields when the designated temperature was reached. The diameter of the nozzle from which a jet stream of high-temperature high-pressure fluid was injected into the low-temperature

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14. Careful SEM-EDS observations suggest that at least 15 and 40 Ols of similar morphologies are present in the high olivine proportions but are more much compact than the latter. Ols and AOAs [A. Hashimoto and L. Grossman, ibid. 51, 1685 (1987)] are clearly distinguished from CAIs in mineral proportions, textures, chemistry, and over all morphologies.

15. Pyroxene is fassaitic to diopside in composition with variable aluminum content. Feldspathic phase is a fine grained mixture of anorthitic plagclase and grossular, which is probably an alteration product of melilitite.

16. Olivines in these chondrules are very Mg-rich [Mg# = 93.3 for both of two], which is comparable to those in Ols, but its oxygen isotopic composition (measured only for Y86009-F) is much less anomalous than the latter, indicating that Mg# alone cannot be a measure of the degree of oxygen isotopic anomalies.


18. D. D. Clayton and co-workers proposed a different model, in which refractory interstellar dust may have longer lifetimes and may retain "cosmic chemical memory" of the earlier stage of galactic chemical evolution, resulting in ~5% excess in 16O on average [K. Liffman and D. D. Clayton, Proc. Lunar Planet. Sci. Conf. 18, 657 (1988); D. D. Clayton, Astrophys. J. 334, 191 (1988).] P. Scowmen, K. Liffman, ibid. 346, 531 (1989). This model may also have difficulties in explaining the uniqueness of the oxygen isotopic anomalies observed in various objects, especially similar anomalies both inside and on the rims of Ols (19).


27. The Yamato-86009 CV3 chondrite sample was supplied by the National Institute of Polar Research, Japan. This work was financially supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (no. 09440183 to H.H.).
chamber was 100 μm, and the injection rate was 8 to 12 ml/min. The chamber had a fluid volume of 78.5 ml, and the temperature was maintained externally at 0°C. The flow rate of the jet stream was adjusted to maintain the pressure of the low-temperature chamber at 24.0 MPa. Accordingly, the turnover time of the whole circulating fluid was 1.0 to 1.3 hours. The downstream vessel was depressurized by inserting a stainless steel tube (0.5 m long and 100 μm in diameter) between the high-pressure low-temperature chamber and the depressurized vessel. For measurements, aliquots of 5 μl were taken out of the depressurized vessel at fixed time intervals.

The reacting chemicals cycled from the low-temperature chamber back to the high-temperature chamber in either 34 or 78 s, which is much shorter than the turnover time of the whole fluid. The cycle time was set by inserting different lengths of tube (800 μm in diameter) between the depressurized vessel and the pressurization pump. It was confirmed that reactants in the different vessels were stirred (11). The major factor determining the cycle time of reactants was the convection of the fluid through the 100-μm tube.

The temperature of the injecting jet stream from the nozzle that was connected to the high-pressure high-temperature chamber was first set at 225°C; the reaction fluid contained 100 mM glycine that was dissolved in pure water with no pH control or added salts. A high-performance liquid chromatography (HPLC) profile of the products revealed that, with time, at least three different oligomers formed: diketopiperazine and the dimer and trimer of glycine (Fig. 2, A and B). We also identified these species with LC mass spectrometry. The initial growth of both the dimer and the trimer was exponential in time. The doubling time was 33 s for the cycle time of 34 s and was 80 s for the cycle time of 78 s (Fig. 2C). The coincidence between the doubling time and the cycle time of reactants traveling the closed path of the flow reactor suggests that both di- and triglycerine formed sequentially, in the sense that the preceding products served as a base for adding monomers one by one as compounds repeatedly traveled the closed path of the reactor.

When 10 mM CuCl₂ was added to the 100 mM glycine solution and the pH was adjusted to 2.5 by HCl at room temperature, higher oligomers were obtained (Fig. 3) in an experiment in which the temperature of the high-pressure high-temperature chamber was set at 250°C at 24.0 MPa and the cycle time was maintained at 34 s. A HPLC profile identified at least four different oligomers: diketopiperazine, diglycine, tetraglycine, and hexaglycine (12). Copper ions were found to help synthesize tetruglycine, as suggested by its exponential initial growth. Even hexaglycine was synthesized after a sufficient amount of tetruglycine was formed.

The fact that di- and triglycerine were synthesized with no detectable amount of tetruglycine in pure water suggests that tetruglycine molecules could be rapidly hydrolyzed into two molecules of diglycine. Two molecules of diglycine could then yield two more molecules of triglycerine when they reentered the reaction region in the high-pressure high-temperature chamber. The initial increment of the yields of triglycerine also suggests that monomeric glycine could aminolyse diketopiperazine to form triglycerine (13).

The presence of copper ions seems to

![Fig. 2](image-url). Time courses of the reaction yields for the reaction fluid containing only 100 mM glycine, with no pH control or added salts. The reactants traveled the closed path of the flow reactor from the high-pressure low-temperature chamber back to the high-pressure high-temperature chamber in cycle times of (A) 34 and (B) 78 s. For reference, the amount of monomeric glycine in the solution is also presented. (C) Time development of the yields of diglycerine for cycle times of 34 and 78 s. The yield was estimated by referring the area of each corresponding HPLC peak to a standard reference of a given concentration. The linearity between the area and the concentration was confirmed. The temperature of the high-pressure high-temperature chamber was set at 225°C.

All samples were analyzed with a Hitachi [L-6300, L-4200, and D-2500] HPLC apparatus with a Shodex Asahipak column (ODP-50) (5 μm by 4.6 mm by 150 mm). The mobile phase consisted of 50 mM KH₂PO₄ and 7.2 mM C₆H₁₃SO₃Na, and its pH was maintained at 2.5 by adjusting the added amount of H₃PO₄. The flow rate of the mobile phase was 0.5 ml/min; this was detected by measuring the absorbance at 195 nm. As standards, glycine and its oligomers up to hexaglycine were purchased from Sigma-Aldrich.

![Fig. 3](image-url). Time courses of the reaction yields for the reaction fluid that contained 100 mM glycine and 10 mM CuCl₂ and was adjusted to a pH of 2.5 by HCl at room temperature. The temperature of the high-pressure high-temperature chamber was set at 250°C at 24.0 MPa. The HPLC conditions were the same as in Fig. 2. For reference, the amount of monomeric glycine (Gly) in the solution is also presented; (Gly)_2 represents diglycerine, (Gly)_4 represents tetraglycine, and (Gly)_6 represents hexaglycine.
have prevented the hydrolysis of tetracyglycine. Tetracyglycine therefore reentered the reaction region and further reacted with a glycine, producing a diglycine, a triglycine, or a diketopiperazine molecule when the amount of tetracyglycine becomes sufficient. The presence of even-numbered oligomers up to hexaglycine and the absence of detectable amounts of both tri- and pentaglycine suggest that the chain elongation proceeds mainly by aminolytic cleavage of diketopiperazine.

As monomers of biological significance, both amino acid and nucleotide molecules can potentially accommodate stepwise polymerization schemes into themselves (2, 3) [for instance, by repeating the cycle of hydrolysis and elongation (4)]. From an evolutionary perspective, a more pressing issue in this regard is how to implement such schemes. Stepwise synthesis of oligoglycine in our flow reactor seems to suggest that submarine hydrothermal vents in the Archean ocean could have readily facilitated the multiplicative oligomerization of these monomers, even in the absence of ribosomes or ribozymes.

References and Notes
9. K. Matsuno, Protobiology: Physical Basis of Biology (CRC Press, Boca Raton, FL, 1989). When a small hot body comes into contact with a large cold body, the temperature of the hot body decreases. In particular, the actual temperature drop is the fastest possible alternative. If the slower process was to take over, the heat flow toward the cold body would increase because of the greater temperature difference between the two bodies. The greater transference of heat energy toward the cold body with a smaller temperature drop of the hot body in time is, however, a self-constradiction. Actualization of the fastest temperature drop is selective in comparison to germinative thermal synthesis at a high temperature.

Oligomeric Structure of the Human EphB2 Receptor SAM Domain

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The sterile alpha motif (SAM) domain is a protein interaction module that is present in diverse signal-transducing proteins. SAM domains are known to form homo- and hetero-oligomers. The crystal structure of the SAM domain from an Eph receptor tyrosine kinase, EphB2, reveals two large interfaces. In one interface, adjacent monomers exchange amino-terminal peptides that insert into a hydrophobic groove on each neighbor. A second interface is composed of the carboxy-terminal helix and a nearby loop. A possible oligomer, constructed from a combination of these binding modes, may provide a platform for the formation of larger protein complexes.

Proteins containing SAM domains include the Eph family of receptor tyrosine kinases (1), diacylglycerol kinases (2), serine-threonine kinases (3). Src homology 2 (SH2) domain–containing adapter proteins (4, 5), ETS transcription factors (6), polyhomeotic proteins (6, 7), and the connector enhancer of KSR (kinase suppressor of ras) (8), among others. The presence of a SAM domain in a wide variety of proteins suggests that, like other signal transduction modules (9), it confers a common function.

Previous studies suggest that SAM domains form SAM homo-oligomers and SAM hetero-oligomers. First, the SAM domain from the ETS transcription factor TEL (TEL-SAM) has been shown to self-associate (10). In many human leukemias, chromosomal translocations render the TEL-SAM domain fused to other proteins including the tyrosine kinase domains of Abelson leukemia virus kinase, platelet-derived growth factor receptor–β, and Janus kinase 2 as well as the transcription factor AML1 (11, 12). TEL-SAM domain oligomerization results in constitutive activation of the protein to which the SAM domain is fused and may cause cell transformation (13). Second, SAM domains from various polycystic group (PcG) proteins, which regulate homeotic gene transcription, also form specific homo- and hetero-oligomers and may be important for generating large PcG protein complexes within the cell (14). Third, the SAM domains of Byr2 and Ste4, proteins that regulate sporulation in the yeast Schizosaccharomyces pombe, form a hetero-oligomer (3, 14–16).

SAM domains also bind to proteins that do not contain SAM domains. The LAR (leukocyte common antigen-related) protein tyrosine phosphatase (PTP) binds to a region of LIP (LAR-interacting protein) that consists of three tandem SAM domains, indicating that SAM domains bind directly to PTPs (17). Other evidence supports a role for SAM domains in PTP binding. Stein et al. reported that binding of low molecular weight PTP (LMPTP) to the EphB1 receptor tyrosine kinase is abrogated by a Y929F mutation (in which Tyr929 is mutated to Phe) in the SAM domain (18). This same mutation also abolished binding of the SH2-containing adapter protein Grb10 (19). These data suggest that phosphorylation of Tyr929 in the EphB1 receptor SAM domain creates a binding site for LMPTP and Grb10 (18). Finally, the PDZ domain of the ras-binding protein AF6 recognizes a peptide that corresponds to the COOH-terminus of the SAM domain in various Eph receptors (20).

Here we report the crystal structure of the SAM domain from the EphB2 receptor. The Eph receptors are the largest family of receptor tyrosine kinases and have been implicated in the regulation of segmentation of the developing brain, retinotectal axon guidance and bundling, angiogenesis, and cell migration (21). All Eph receptors contain a SAM domain at their COOH-terminus.

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