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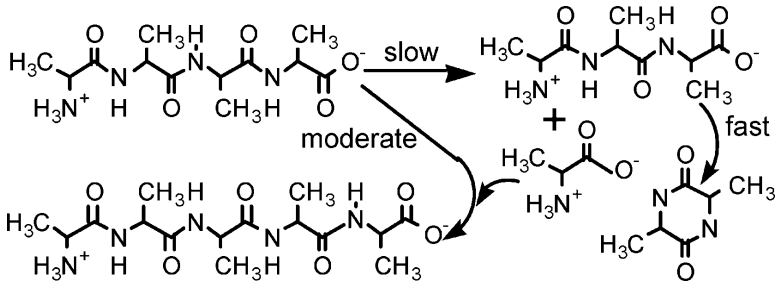

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Consecutive Elongation of Alanine Oligopeptides at the Second Time Range under Hydrothermal Conditions Using a Microflow Reactor System

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The prebiotic simulations of the submarine hydrothermal vent system suggest that hydrothermal reactions have played important roles for the emergence of life on the primitive earth.[1−5] However, the yields of the direct formation of oligopeptides from amino acid monomers in these simulations were typically 0.1−1%.[4,5] Thus, it should not be straightforwardly concluded that the proteinlike molecules have been easily accumulated through the primitive hydrothermal vent systems. On the other hand, from the viewpoint of the demand for environmentally harmless organic synthesis processes, the efficient preparation of oligopeptides without organic solvent is attractive. Recently, we have established a new microflow reactor system and investigated the reaction behavior of amino acids under hydrothermal conditions.[6] These kinetic studies have shown that large amounts of diketopiperazine (DKP) formed from amino acid monomers.[4−8] This pathway is regarded as a stumbling block from the viewpoint concerning either the prebiotic formation of oligopeptides in hydrothermal systems or the synthetic organic chemistry of oligopeptides in aqueous solution. To solve this problem, the reaction behaviors of alanine oligopeptides longer than L-alanyl−L-alanine ($(Ala)_2$) have been investigated using starting materials L-alanyl−L-alanyl−L-alanine ($(Ala)_3$), L-alanyl−L-alanyl−L-alaniyl−L-alanine ($(Ala)_4$), and L-alanyl−L-alanyl−L-alaniyl−L-alaniyl−L-alanine ($(Ala)_5$) since $(Ala)_2$ is immediately converted to DKPs.

The reactions of alanine oligopeptides were monitored at 250−310 °C at 10−15 MPa within 0.29−137 s using the microflow reactor system[6,9,10] with and without Ala monomer. The reactions were monitored by two different HPLC methods on a chiral column (Crown Pack, Daisel Co.) using a buffer of $HClO_4$ at pH 1.5 and on a reversed-phase column (CAPCELLPAK C18, Shiseido) using a buffer containing 50 mM NaH_2PO_4 and 7.2 mM $CH_3(CH_2)_5SO_3Na$ at pH 2.5. The products were characterized by comparing the HPLC retention times of the products with those of authentic reagents. Some of the products were isolated and identified by MALDI-MASS spectrometry. Although the main products from these starting materials were DKP, the disappearance of $(Ala)_4$ and $(Ala)_5$ was much slower than that of $(Ala)_2$ and $(Ala)_3$. The tubing materials do not affect the reactions at these temperatures. The reactions of $(Ala)_4$ and $(Ala)_5$ were complete within 50−100 s at 275 °C (Figure 1). The products from $(Ala)_4$ and $(Ala)_5$ showed that the racemization occurred during the formation of DKP from $(Ala)_4$ and $(Ala)_5$. Additionally, it was surprising that a small amount of $(Ala)_5$ was detected in the products from $(Ala)_4$, which was characterized using the two different HPLC methods. Likewise, an HPLC peak possessing longer retention time than that of $(Ala)_5$ on the reversed-phase column was observed in the reaction of $(Ala)_5$. This was isolated through the reversed-phase HPLC and dialyzed to run MALDI-MASS analysis on a Shimadzu KOMPACT MALDI2. It was confirmed that the product was $(Ala)_6$ (444.3 [$M^{+\bullet}$], 468.5 [M + Na]). Moreover, some unknown products were

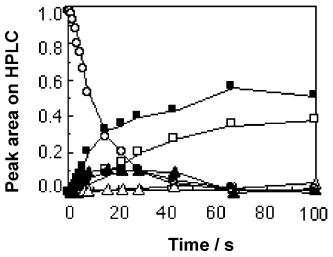
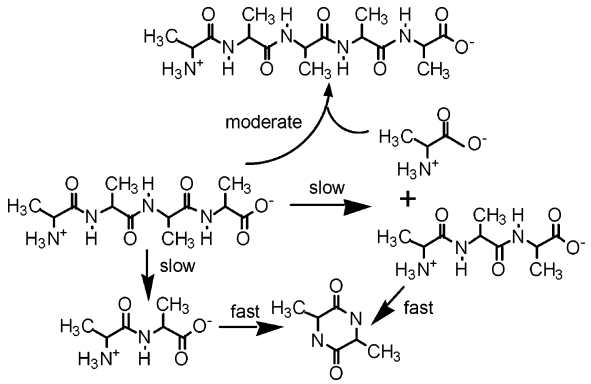


Figure 1. Reaction curves for the reaction of $(Ala)_4$: [NaCl] = 0.1 M, $[MgCl_2]$ = 0.05 M, $[(Ala)_4]$ = 1 mM, initial pH of 7.0 at 25 °C, 275 °C, 10 MPa. ○, $(Ala)_4$; ▲, unknown; ●, $(Ala)_5$ isomers; □ and ■, DKP; △, alanine.

Scheme 1. Estimated Pathways of the Degradation and Elongation of $(Ala)_4$ under the Hydrothermal Conditions



observed at retention times longer than that of $(Ala)_6$ in the products at different reaction time and temperature. MASS analyses showed that the products possess molecular weights of 513.6, 727.7, and 941.5, which are consistent with those of $(Ala)_7$, $(Ala)_{10}$, and $(Ala)_{13}$, respectively. On the other hand, the reaction of $(Ala)_3$ was complete within 10 s at 275 °C, where a single DKP isomer was detected, but the extent of Ala was very low. In addition, it was unexpected that the disappearance of $(Ala)_3$ was faster than that of $(Ala)_2$. The further exposure of the reaction solution of $(Ala)_3$ or $(Ala)_2$ at 250−290 °C for 20−100 s formed DKP diastereomers.

First-order rate plots of the disappearance of $(Ala)_3$, $(Ala)_4$, and $(Ala)_5$ are consistent with pseudo-first-order processes, where the apparent rate constants (k_{app}) decreased in the order $(Ala)_3$ ($0.88 \pm 0.04\ s^{-1}$) > $(Ala)_2$ ($0.25 \pm 0.08\ s^{-1}$) ≫ $(Ala)_4$ ($0.066 \pm 0.002\ s^{-1}$) > $(Ala)_5$ ($0.046 \pm 0.001\ s^{-1}$) at 275 °C. Since the decarboxylation of amino acids is slower than the formation of DKP, the decarboxylation can be ignored in these systems.[11] In addition, it is estimated from thermodynamic data that the pH of the alanine oligopeptide solutions prepared at 25 °C changes to around 4.0 at 250−290 °C, where alanine oligopeptides exist as zwitterions.[12] It is reasonable that DKP forms by the internal aminolysis of $(Ala)_3$ since the disappearance of $(Ala)_3$ to form DKP was faster than that

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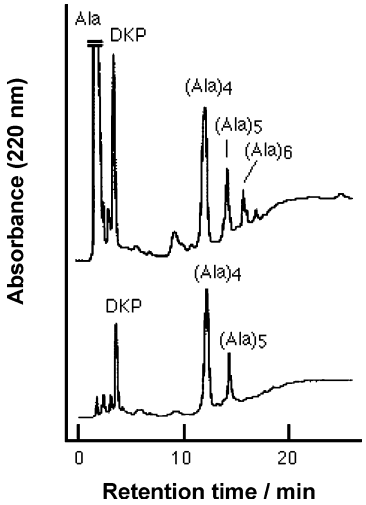

Figure 2. HPLC charts for the hydrothermal elongation of $(Ala)_4$ in the presence and absence of an excess amount of Ala monomer. Reaction conditions are the same as those shown in Figure 1. Reaction time: 18.2 s, 15 MPa. Top: [Ala] = 0.1 M. Bottom: No Ala was added. HPLC was performed using a CAPCELLPAK C18.

of $(Ala)_2$.[6,13] Besides, the reaction curves of $(Ala)_4$ and $(Ala)_5$ suggest that an indirect formation of DKP occurred via $(Ala)_2$ and/or $(Ala)_3$ formed by the cleavage of the peptide bonds of $(Ala)_4$ and $(Ala)_5$. This is in contrast to a previous study on hexapeptide, in which DKP was assumed to form by the internal aminolysis.[13] The likely reaction pathways of $(Ala)_4$ in the present system are expressed in Scheme 1; the reaction of $(Ala)_5$ should follow the similar pathways. Presumably, the consecutive elongation occurred from $(Ala)_4$ and $(Ala)_5$ with Ala monomer, which should be formed by the cleavage of the oligopeptides since the extent of $(Ala)_5$ is greater than that of $(Ala)_6$.

On the basis of Scheme 1, the elongation would occur more efficiently if we design the elongation reaction from $(Ala)_4$ or $(Ala)_5$, as shown in the following equation.

$$(Ala)_4 + \text{Ala (excess)} \rightarrow (Ala)_5$$

The reactions involving $(Ala)_3$, $(Ala)_4$, or $(Ala)_5$ with additional Ala monomer were examined. As we expected, the elongation from $(Ala)_4$ to longer oligopeptides was indeed enhanced (Figure 2). A notable amount of $(Ala)_5$ and $(Ala)_6$ was observed in the reaction of $(Ala)_4$ with Ala monomer, where the efficiency of the elongation was >10%. This is about 10−100 times greater than that obtained in previous simulation studies. The extent and the reaction rate of the elongated oligopeptides increased with increasing Ala monomer. Naturally, the elongation to $(Ala)_6$ proceeded in the reaction of $(Ala)_5$ with Ala. Although the possibility of the direct coupling of $(Ala)_4$ or $(Ala)_5$ to form higher oligopeptides was examined, no evidence of the direct coupling was detected. In contrast to the cases of $(Ala)_4$ and $(Ala)_5$, the elongation did not proceed at all in the reaction of $(Ala)_3$ in the presence of Ala monomer.

This elongation reaction is more efficient than the previous hydrothermal reactions using amino acid monomers, although the efficiency may be regarded as moderate compared with that of the conventional organic syntheses using organic solvent. Furthermore, the maximum conversion from $(Ala)_4$ to $(Ala)_5$ was observed at 11−18 s at 275 °C, but the half-life of the racemization of $(Ala)_4$ was 29 s; this is consistent with previous studies.[14] This fact indicates that the racemization of oligopeptides only partially proceeds during the elongation. At the same time, the investigation of a possibility of the hetero- and/or homochiral elongation with L- and D-Ala to $(Ala)_4$ would be notably important as a future subject.

This study elucidated for the first time that the elongation of $(Ala)_4$ and $(Ala)_5$ is possible in the presence of Ala monomer. The low efficiency of the formation in the previous simulation studies of the hydrothermal vent system is due to the termination reactions of the formation of DKP from $(Ala)_3$ as well as $(Ala)_2$. This type of hydrothermal elongation process on the primitive earth might have been possible by the continuous supplementation of amino acid monomers.[15] Furthermore, this elongation reaction would be applicable for the consecutive synthesis of oligopeptides within aqueous solutions combined using the hydrothermal microflow reactor system.

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Supporting Information Available: Reaction curves of $(Ala)_3$, $(Ala)_4$, and $(Ala)_5$ without and with Ala monomer. This material is available free of charge via the Internet at http://pubs.acs.org.

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