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Polypeptide Formation from Asparagine-Containing Dipeptides in Aqueous Solution upon Heating

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Heating the aqueous solutions of asparagine-containing dipeptides in which asparagine locates at their C-terminal gave sequential polypeptides having the molecular weight up to 3000-5000.

Although many reports on peptide formation reactions in the process of chemical evolution have been published,[1-6] how polypeptides formed on the primitive earth is still under discussion.[1] Mainly five types of simulational experiments have been carried out.[1-6] Those are the heating reaction of solid amino acids(1),[2] the reactions of activated amino acids(2),[3] the polycondensation of amino acids in aqueous media in the presence of condensing agents(3),[4] the polycondensation of HCN or aminoacetaldehyde(4),[5] and the heating reaction of aqueous solution containing amino acids(5).[6] The experiments involved in the first category have been well investigated in the relation with proteinoid microsphere.[2] The problem in the reaction of activated amino acids[3] is the stability of the activated compounds such as aminoacyl adenylates. And the simulational experiments belonging to the categories (3) and (4) have afforded only shorter than penta peptides.[4,5] On the other hand, asparagine gives polyaspartic acid in aqueous solution upon heating,[6] because asparagine is an amino acid having amide group which would be a leaving group for peptide bond formation.

In this paper, we wish to report the formation of polypeptides from aspargine-containing dipeptides in aqueous solution upon heating at the constant temperature near 100°C at the pH 7 to 8. Such conditions are considered to be commonly found in the primitive hydrosphere.[1] Peptide substrates (glycyl-L-asparagine(Gly-L-Asn, **1**), L-alanyl-L-asparagine(L-Ala-L-Asn, **2**)) were prepared by the hydrogenolysis of Z-Gly-L-Asn(Z:benzyloxycarbonyl) and Z-L-Ala-L-Asn, which were obtained by the

coupling of L-Asn with N-hydroxysuccinimide esters of Z-amino acids(Z-Gly and Z-L-Ala).

The aqueous solutions of peptide substrates **1**(1.0 ml, 1.0 M), **2**(1.0 ml, 1.0M), and asparagine(**3**:5.0 ml, 1.0 M) were heated at 100, 110, 120 °C. All the reaction solutions heated at the constant temperature for the constant hours were analyzed by amino acid analyzer. And the remaining reaction solution was loaded into a Sephadex G-25 column. Higher molecular weight fractions than authentic $(Pro\text{-}Pro\text{-}Gly)_5$ (Mw=1274, Peptide Institute, Inc.) were pooled and lyophilized to give white amorphous powder.

Figure 1 shows the time courses of amino acids, Ala-Asn and ammonia during the heating reactions of substrates **2** and **3** at 120 °C. In all reactions, the concentration of Ala-Asn decreased and that of ammonia increased with the reaction proceeded. The almost same results were obtained in the reactions of substrate **1**.

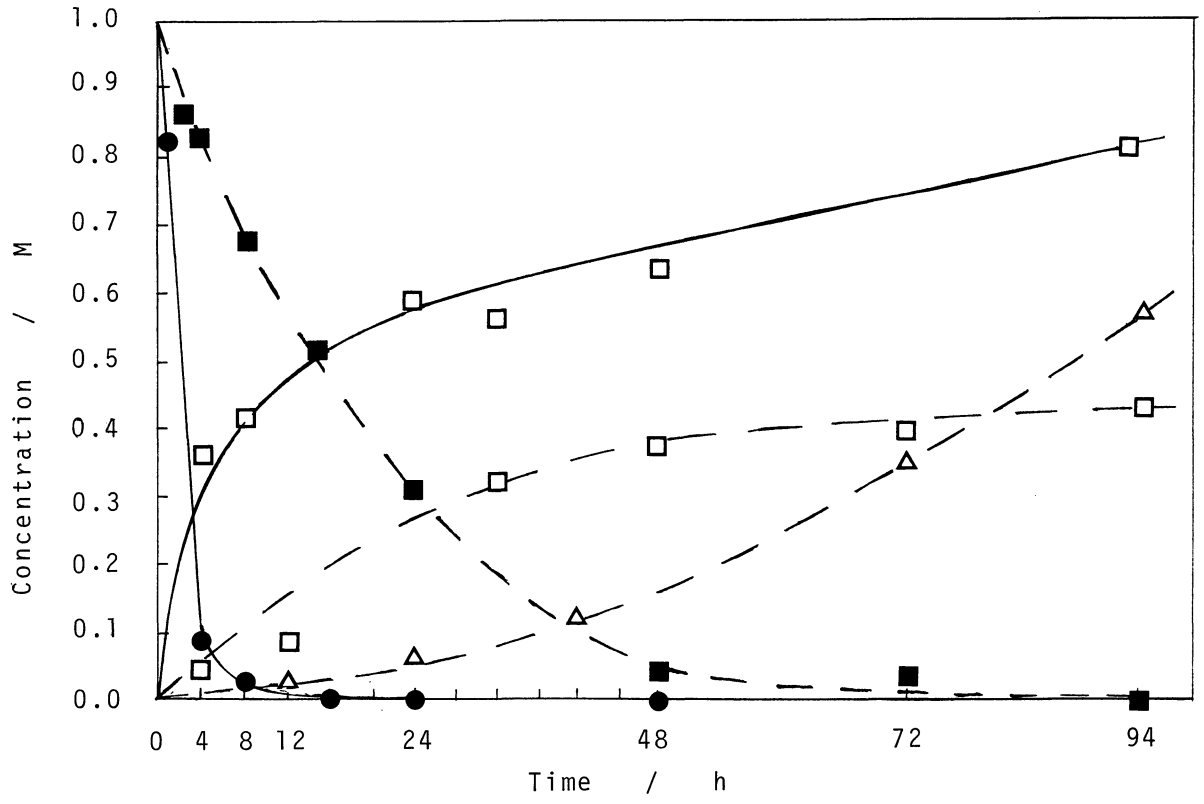

Fig. 1. Time-course of Ala-Asn(●), Asn(■), NH_3(□), and Asp(△) during the reactions of Ala-Asn(———) and Asn(– – – –) at 120°C.

This observation indicates that the peptide formation reactions can be explained by the deamidation mechanism[7,8] as shown in Fig. 2. And the rate of decrease of substrate **2** was rather faster than that of substrate **3**. The reaction rate of deamidation depends on the protonation of amino group

as the nucleophile. Amino groups of dipeptide substrates **1,2** would be less protonated than asparagine, because the amino group of peptide substrates are farer from the carboxylic group in those molecules comparing with aspargine(3).

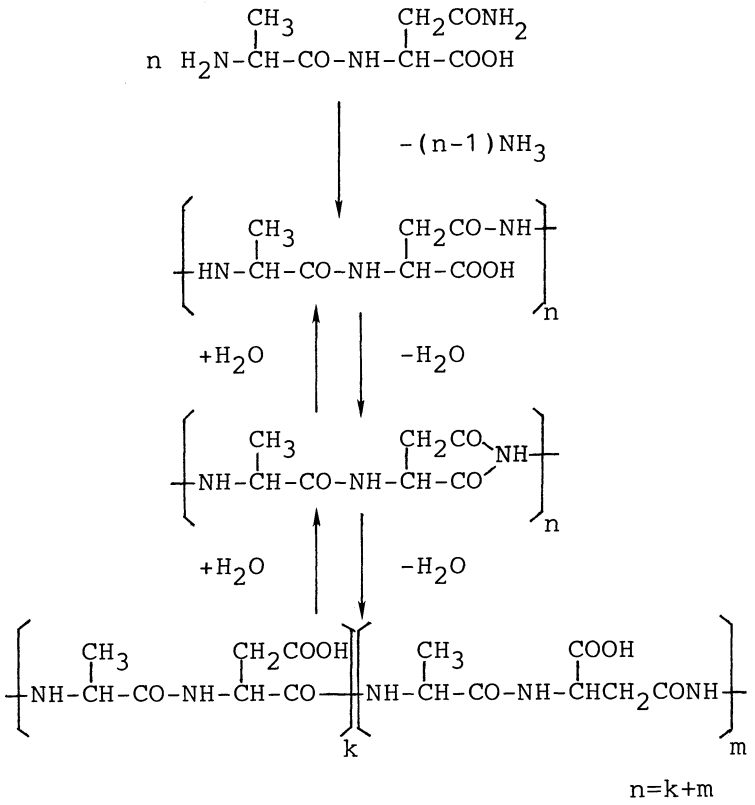

Fig. 2. Amidation mechanism for the peptide bond formation from Asn-containing dipeptides in aqueous solution upon heating.

Table 1 shows the yield of the purified higher molecular peptides and the amino acid recovery after hydrolysis. The yields of the higher molecular peptides were 13 to 22 %. The molecular weight of the purified polypeptides was estimated in the range of 3000 to 5000. The amino acid composition of the polypeptides obtained from substrates **1,2** was almost 1:1. The IR of the purified peptides showed the absorption bands(1710(COOH), 1630(amide I), 1540 cm^{-1}(amide II)) due to acidic polypeptide. These results indicate that the polypeptides have a sequential structure as $(AA\text{-}Asp)_n$. This would be the first example of the sequential polypeptide formation in aqueous solution upon heating. The formation of suquential polypeptide upon heating in this study shows a possible passway for the organized polypeptide formation in the primitive hydrosphere.

Table 1. Formation of sequential polypeptides from Asn-containing dipeptides in aqueous solution upon heating

Substrate	Temp /°C[a)]	Time/h[b)]	Yield/mg[c)] (%)[d)]	AA composition[e)]	
				Gly or Ala	Asp
1	120	4	22(13)	0.95	1.00
1	120	8	36(21)	0.93	1.00
1	120	16	23(13)	0.93	1.00
2	100	12	24(13)	1.08	1.00
2	110	8	36(21)	1.05	1.00
2	120	8	41(22)	1.08	1.00

a) Reaction temperature.
b) Reaction time.
c) Yield of purified higher molecular peptide.(The molecular weight was estimated as 3000-5000 by Sephadex G-50F).
d) Mole per cent of (AA-Asp) residue to the initial substrate.
e) Amino acid composition.

Aspargine-containing peptides may have formed from Asn which is supposed to be a prebiotic amino acid[9)] and other amino acids in the presence of condensing agents, and then polymerized to sequential polypeptides. Thus, the Asn residue may play a role like a kind of glue for the peptide bond formation.

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