DIKETOPIPERAZINE-MEDIATED PEPTIDE FORMATION IN AQUEOUS SOLUTION

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Abstract. Though diketopiperazines (DKP) are formed in most experiments concerning the prebiotic peptide formation, the molecules have not been paid attention in the studies of chemical evolution. We have found that triglycine, tetraglycine or pentaglycine are formed in aqueous solution of glycine anhydride (DKP) and glycine, diglycine or triglycine, respectively. A reaction of alanine with DKP resulted in the formation of glycylglycylalanine under the same conditions. These results indicate that the formation of the peptide bonds proceeds through the nucleophilic attack of an amino group of the amino acids or the oligoglycines on the DKP accompanied by the ring-opening.

The formation of glycine anhydride, di-, tri- and tetraglycine was also observed in a mixed aqueous solution of urea and glycine in an open system to allow the evaporation of ammonia. A probable pathway is proposed for prebiotic peptide formation through diketopiperazine on the primitive Earth.

1. Introduction

The process of prebiotic peptide formation is one of the most important subjects in the study of chemical evolution, because peptides are expected to have some catalytic activities to promote and to accelerate further evolution of prebiotic organic compounds. A number of experiments concerning prebiotic peptide formation have been performed under various conditions with amino acids, though some of which seem to have been possible only at special areas on the primitive Earth, or by the use of amino acid derivatives or activated amino acids (Harada and Fox, 1958; Fox and Harada, 1960; Oro and Guidry, 1960, 1961; Paecht-Horowitz and Katchalsky, 1967; Lewinsohn *et al.*, 1967; Sawai and Orgel, 1975; Nooner *et al.*, 1977; Weber and Orgel, 1978, 1979a, b; White and Erickson, 1980; Yanagawa *et al.*, 1984; Yanagawa and Kojima, 1985). However, it seems more likely to us that the chemical evolution might have taken place in the primitive oceans as a more universal place where peptides were formed from amino acids with an aid of various condensing agents and were accumulated gradually against their hydrolysis under mild conditions. Several simulation experiments were performed from such a viewpoint in aqueous solutions containing condensing agents (Steinman *et al.*, 1964, 1966; Rabinowitz *et al.*, 1969; Chung *et al.*, 1971; Rabinowitz and Hampei, 1978; Hawker and Oro, 1981; Sakurai and Yanagawa, 1984; Yamanaka *et al.*, 1988).

Diketopiperazines (DKPs) are easily formed in most experiments concerning prebiotic peptide formation. However, the molecules have not been paid attention

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in a study of chemical evolution, but their formation has been considered to be an obstacle for the peptide chain elongation beyond dipeptide (Brack *et al.*, 1976; Weber and Orgel, 1978, 1979a). We have found that the reactions of DKP with amino acids or peptides in aqueous solution resulted in the formation and chain elongation of oligopeptides. We propose here a novel process for prebiotic peptide synthesis by way of DKP.

2. Material and Method

Glycine, urea and sodium cyanate were purchased from Wako Pure Chemical Co. (Japan), L-α-alanine from Nakarai Chemical Co. (Japan), $(Gly)_2$*, $(Gly)_3$, $(Gly)_4$ and glycine anhydride from Tokyo Kasei Kogyo Co. Ltd. (Japan) and $(Gly)_5$, $(Gly)_6$, glycylglycyl-L-alanine (gly-gly-ala) and L-alanyl-glycylglycine (ala-gly-gly) from Sigma Chemical Co.

Oligopeptides were analyzed by HLPC with ninhydrin reaction system described previously (Yamanaka *et al.*, 1988), which could separate oligoglycins up to at least $(Gly)_6$ and gly-gly-ala, ala-gly-gly, glycine, alanine and $(Gly)_2$ one another. DKP (glycine anhydride) was analyzed by HPLC (ODS column: 4 × 250 mm, detection: 220 nm, flow: H_2O 1.0 mL min^{-1}). The DKP-fraction was partially hydrolyzed (0.5N HCl, 100 °C, 30 min) and the quantity of resulting $(Gly)_2$, which was detected by the HPLC-ninhydrin reaction system, was consistent with that detected by 220 nm.

3. Results

3.1. Formation and chain elongation of oligoglycines by a reaction of glycine or oligoglycines with DKP in aqueous solution

Aqueous solutions containing 0.5 M DKP and 0.5 M Gly, $(Gly)_2$, or $(Gly)_3$ were heated at 90 °C (pH 4–9), and after 5, 10, and 15 hr an aliquot of each solutions was subjected to HPLC with a ninhydrin reaction system. The main products except $(Gly)_2$, which was formed by hydrolysis of DKP, were $(Gly)_3$, $(Gly)_4$ and $(Gly)_5$, respectively. Relatively smaller peaks corresponding to the further reaction products of them with DKP were also found, for example, when Gly or $(Gly)_3$ were used as nucleophile, $(Gly)_4$ were observed in the same order with that in a control experiment (Section 3.2). Thus, the main reaction product with DKP could be generally written as DKP + $(Gly)_n \rightarrow (Gly)_{n+2}$. The experimental results are given in Figures 1-a, b, c. Figure 2 shows a tendency that the larger the number of n, the higher the yield of the resulting peptide.

3.2. A control experiment with DKP alone

As a control experiment for the experiment 3.1, an aqueous solution of DKP (0.5

* $(Gly)_n$ means n-degree polymer of glycine.

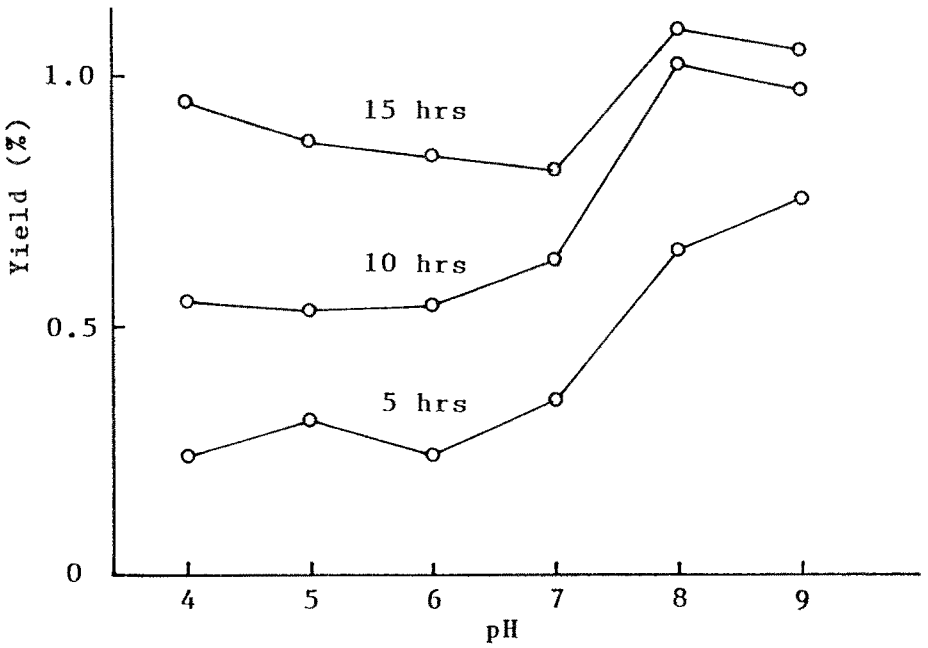
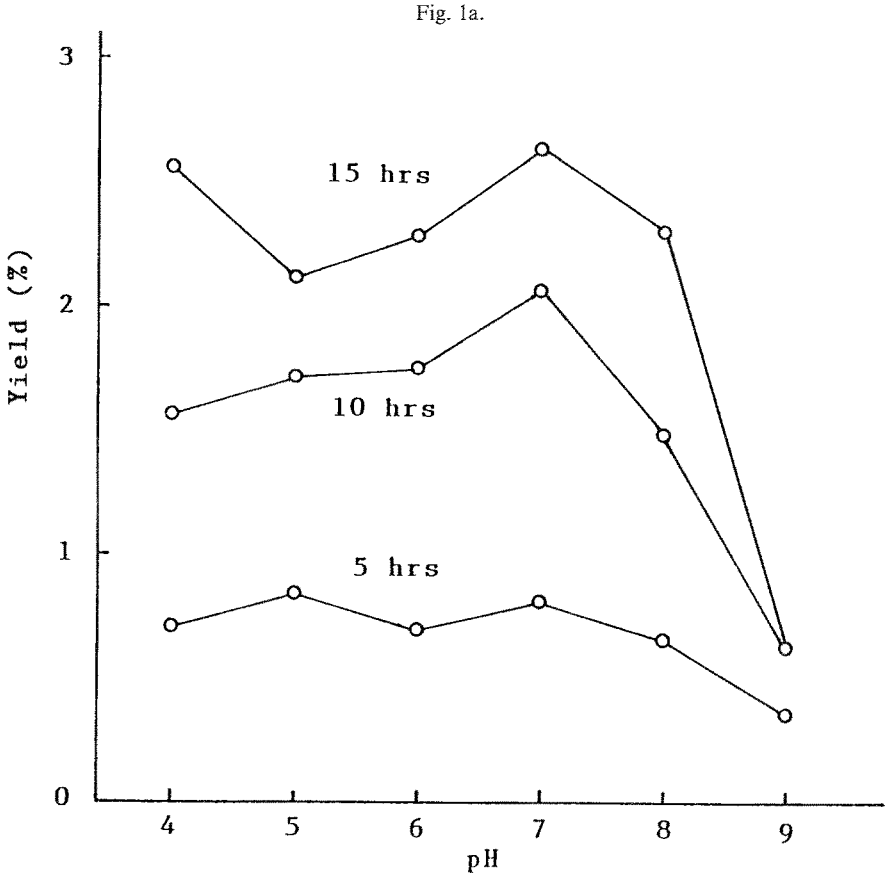

1.0
0.5
0
Yield (%)
15 hrs
10 hrs
5 hrs
4
5
6
7
8
9
pH

Fig. 1a.


3
2
1
0
Yield (%)
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pH

Fig. 1b.

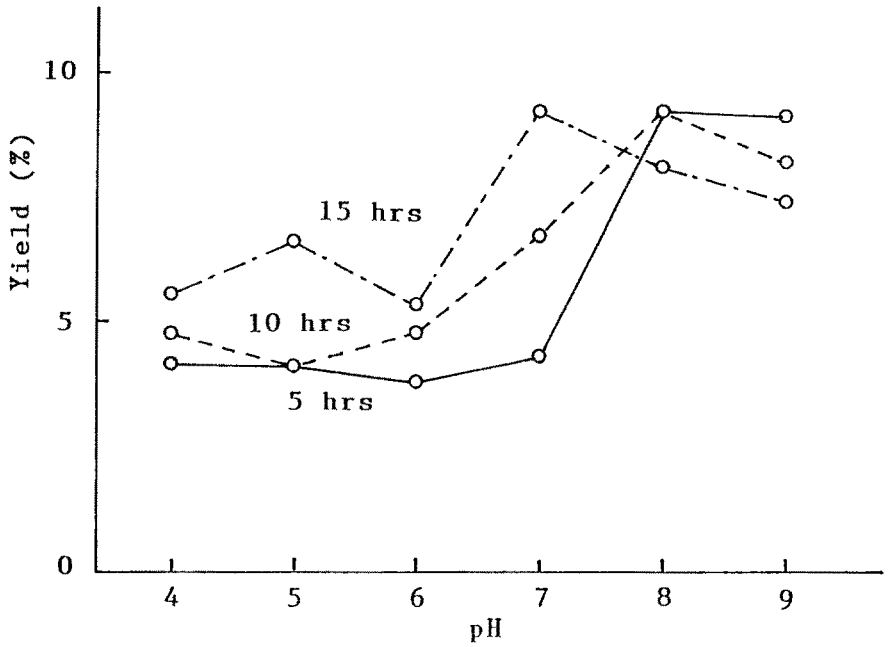

Fig. 1c.

Figs. 1a–c. pH and time dependence of the yield of $(Gly)_{n+2}$ in the reactions: DKP (0.5 M) + $(Gly)_n$ (0.5 M) → $(Gly)_{n+2}$ at 90 °C in aqueous solution. Yield means percent of the concentration of the produced $(Gly)_{n+2}$ to that of the starting $(Gly)_n$. (a) n=1, (b) n=2, (c) n=3

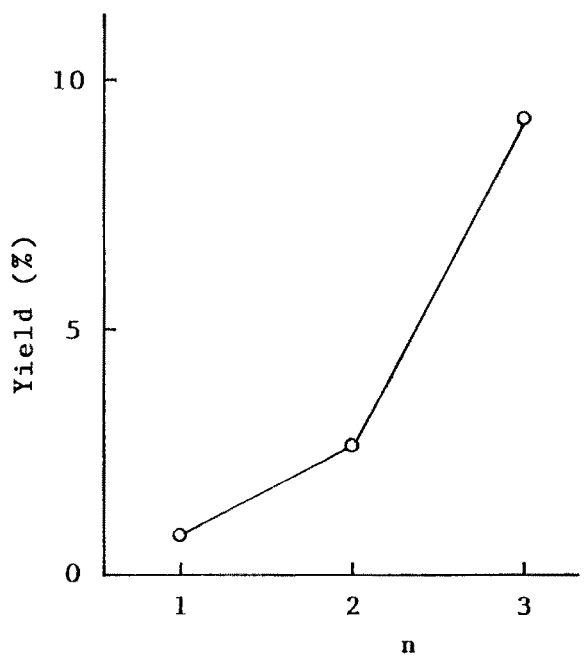

Fig. 2. Dependence of the yield of $(Gly)_{n+2}$ on n in the reactions: DKP + $(Gly)_n$ → $(Gly)_{n+2}$ at pH 7, 90 °C after 15 hr.

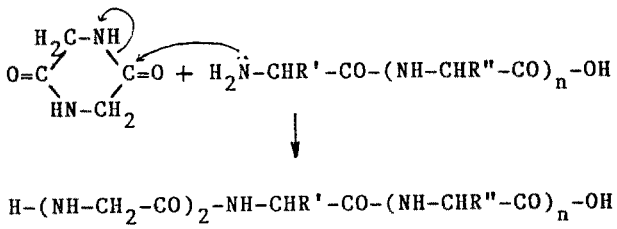

Fig. 3. A probable mechanism of the reactions shown in Figure 1.

M) was treated under the same conditions at pH 7. After heating for 15 hr at 90 °C, Gly, $(Gly)_2$ and $(Gly)_4$ were formed in 0.07%, 8.9% and 0.06% yields (% DKP converted to each of them), respectively.

3.3. A REACTION OF ALANINE WITH DKP

We have also investigated a reaction of alanine with DKP under the same conditions

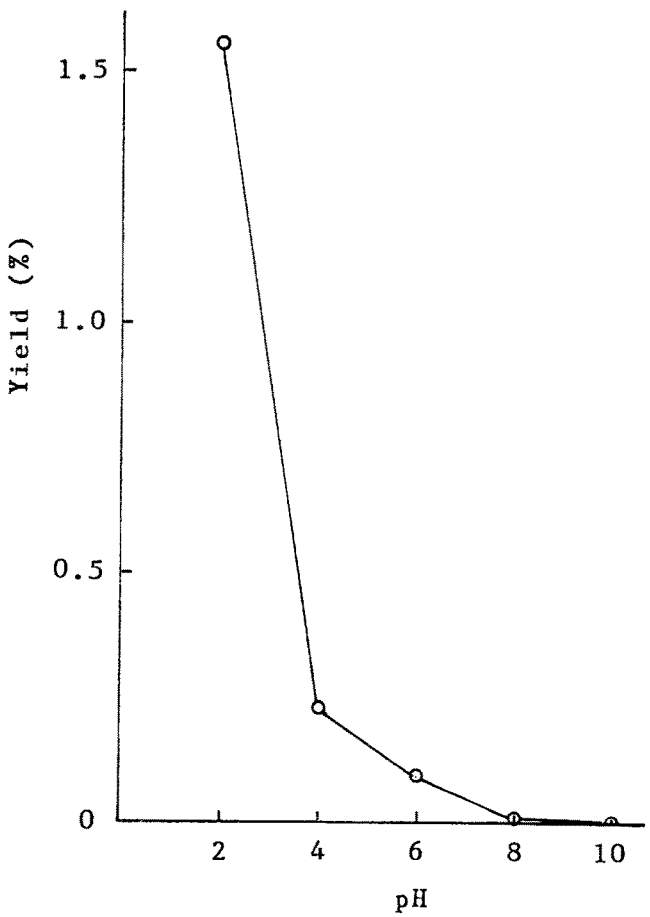

Fig. 4. pH dependence of the yield of DKP formed by the cyclization of $(Gly)_2$ (0.5 M) with NaOCN (0.5 M) in aqueous solution at 39 °C after 3 hr.

to elucidate the reaction mechanism. The resulting tripeptide was Gly-Gly-Ala but not Ala-Gly-Gly. Thus, the reactions seem to proceed through the nucleophilic attack of an amino group of amino acid or oligoglycine on DKP accompanied by its ring-opening (Figure 3).

3.4. Formation of DKP from $(Gly)_2$ and cyanate in aqueous solution

DKP was formed in a mixture of 0.5 M $(Gly)_2$ and 0.5 M sodium cyanata at 39 °C (Figure 4). A probable reaction mechanism is shown in Figure 5.

3.5. Formation of DKP and oligoglycines from Gly and urea in aqueous solution

Since urea is most readily produced in a variety of chemical evolution experiments, it may have played important roles in chemical evolution. Sakurai and Yanagawa (1984) reported the formation of dipeptides from amino acids and urea in aqueous solution and discussed its reaction mechanism. Yamagata *et al.* (1984) showed that urea worked as a condensing agent for the formation of diglycine from glycine and also for the phosphorylation of adenosine in aqueous solution.

Urea dissociates to ammonia and cyanate, which works as an effective condensing agent as shown above, in very low concentration in equilibrium. However, if the resulting ammonia is removed, the equilibrium would be shifted to increase the concentration of cyanate (urea → HN=C=O + $NH_3^{\uparrow}$). Therefore, the condensation reactions using urea should be much more accelerated in an open system to allow rapid escape of ammonia.

An experiment using a beaker was performed supplying water continuously to compensate the evaporated water. The results are shown in Figure 6.

3.6. Formation of DKP and oligoglycines from diglycine and urea in aqueous solution

An experiment similar to that in the section 3.5 was performed using diglycine as the starting material. As expected, the formation of DKP, $(Gly)_4$ and $(Gly)_6$ was observed (Figure 7).

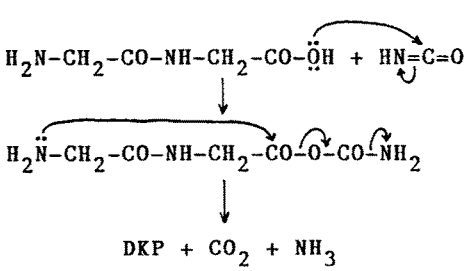

Fig. 5. Mechanism of the cyclization of $(Gly)_2$ to form DKP shown in Figure 4.

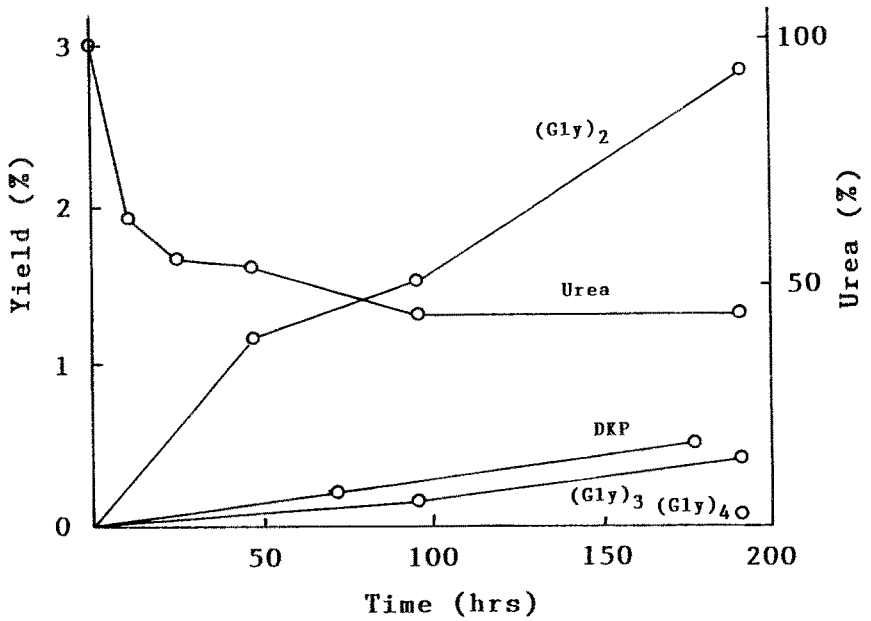

Fig. 6. Formation of DKP and oligoglycines in the aqueous solution of 0.5 M Gly and 0.5 M urea, and consumption of urea at approximately pH 7 at 90 °C.

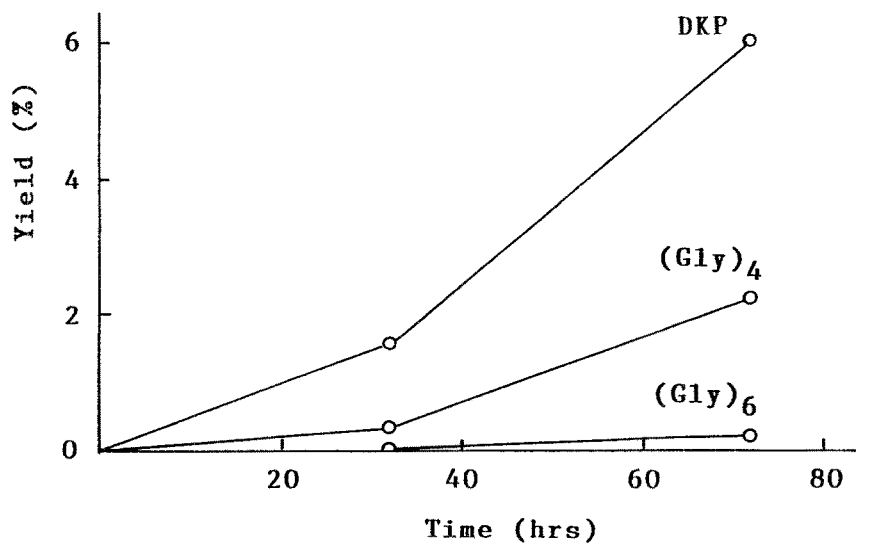

Fig. 7. Formation of DKP and oligoglycines in the aqueous solution of 0.5 M $(Gly)_2$ and 0.5 M urea at approximately pH 5 at 90 °C.

4. Discussion

Dipeptides are transformed into DKP by intramolecular dehydration condensation reaction in the presence of condensing agents in aqueous solution. The intramolecular reaction proceeds generally much faster than intermolecular condensation between the dipeptide and other amino acids or peptides, because of the short distance between the amino group and the carboxyl group in the dipeptides and also of stereochemical feasibility to form DKP. Since DKPs have been considered to be chemically inactive and useless compounds, their formation has been believed to be an unavoidable obstacle for the elongation of peptide chain beyond dipeptide.

However, we have found that DKP is not so stable molecule, but it serves as a probable intermediate for prebiotic peptide formation, and that the molecule provides internally the free energy necessary to form the peptide bond.

There are two possible conformations of peptide bond, one is of trans-form and another of cis-form. Peptide bonds in linear peptides hold generally the conformation of the trans-form (Elmore, 1968). This means that the trans-form would be in a lower energy state than the cis-form. However, DKP is forced to hold cis-form of peptide bond, because of the rigid ring structure. Therefore, it is suggested that the peptide bonds in DKP would have higher energy than the trans-form peptide bonds in linear peptides (Elmore, 1968). This might be supported by the fact that poly-L-proline I, which contains only cis peptide bond, are converted to all trans form II in aqueous solution (Torchia and Bovey, 1971). Thus, the ring-opening of DKP could supply the free energy necessary to form a new peptide bond. DKP may be referred to a building block stored energy for the peptide formation.

Sakurai and Yanagawa (1984) have discussed the mechanism of the dimerization of amino acids through their carbamylation by urea in aqueous solution. The dimerization reaction proceeds in rather high rate under alkaline conditions, but the elongation of dipeptides would be almost impossible under the basic conditions because most of the dipeptide would be easily converted to inactive carbamyldipeptide by urea. On the contrary, DKP is easy to form under acidic condition (Figure 4). Since DKP and the resulting peptides are unstable under the acidic conditions, consequently, neutral pH region seems to be the most suitable for the peptide formation.

A probable pathway of the prebiotic peptide formation can be considered as follows. The peptide formation is initiated by the dimerization of amino acids in the presence of urea according to the mechanism proposed by Sakurai and Yanagawa (1984), followed by its cyclization to form DKP. Finally, the DKP is incorporated into a peptide by its ring-opening. It is noteworthy that the larger the number n of $(Gly)_n$ in the reaction with DKP, the higher the yield of resulting peptide as shown in Figure 2. Such tendency would have accelerated the growth of peptides in the chemical evolution, as we have already discussed in the previous reports (Yamagata *et al.*, 1980, Yamanaka *et al.* 1988).

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