# 176. Influence of Imidazole and Hydrocyanic Acid Derivatives on the 'Possible Prebiotic' Polyphosphate Induced Peptide Synthesis in Aqueous Solution

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*Summary*

Glycine in aqueous solutions of trimetaphosphate or linear polyphosphate at pH adjusted to 8.1–9.0, is condensed at room temperature to diglycine and very small amounts of triglycine. The addition of imidazole increases the yield of triglycine by a factor of almost 10; supplementary addition of magnesium ion does not increase this effect. On the contrary to what has been observed at pH 11.5–12.0, the addition of sodium cyanide or cyanamide at pH 8.1–9.0 diminishes strongly the yield of triglycine and to a lesser degree that of diglycine. The prebiotic significance of the condensation of amino acids in aqueous solutions of polyphosphates in the presence of imidazole is discussed.

We have shown that linear and cyclic polyphosphates condense amino acids into dipeptides (and occasionally very small amounts of tripeptides) with yields of up to 40% in aqueous solutions at room temperature and at pH values compatible with those of primitive oceans [1]. The best results are obtained with trimetaphosphate but the reaction proceeds already, though with low yields, with pyrophosphate [1].

Using a number of linear polyphosphates ('polyphosphate glasses', **1**) with a known average chain length $n$ as well as trimetaphosphate (**2**), we can draw the conclusion that the yield of dipeptide calculated per phosphorus atom increases with the chain length $n$ (see *Table 1*); the yield has a maximum using trimetaphosphate. This result is not surprising since the ultimate products of the thermal condensation of dihydrogenphosphates are the metaphosphates, particularly trimetaphosphate [2]; furthermore, the properties of linear polyphosphates **1** become closer to those of trimetaphosphate **2** when $n$ is large, *i.e.* when the ratio $(n-1)/n$ is close to 1 [2].

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Table 1. *Diglycine formation from 0.1M aqueous solutions of glycine in the presence of linear polyphosphates $NaHPO_3$-$(OPO_2Na)_{n-2}$-$OPO_3HNa$ or trimetaphosphate $(NaPO_3)_3$ at 70° and at pH 6.5–8*[a])

| Polyphosphate *n* | Concentration of polyphosphate | | Yield of diglycine | |
|---|---|---|---|---|
| | *c*[b]) | Number of P-atoms per molecule of glycine | Relative to glycine % | Per P-atom % |
| 2 (pyrophosphate) | 0.1 | 2 | 0.3 | 0.15 |
| 3 (tripolyphosphate) | 0.1 | 3 | 0.6 | 0.20 |
| 5.5 | 0.069 | 3.80 | 2.9[c]) | 0.76 |
| 11.5 | 0.034 | 3.91 | 3.2[c]) | 0.82 |
| 18 | 0.022 | 3.96 | 7.3[c]) | 1.84 |
| 26 | 0.015 | 3.90 | 9.2[c]) | 2.36 |
| Trimetaphosphate | 0.1 | 3 | 14.8[c]) | 4.93 |

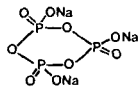
[a]) pH adjusted to 8 with concentrated ammonia every few hours; 6.5 is the lowest pH attained during the reaction.
[b]) *c* in mol/l.
[c]) Results taken from previous works [1].

$$n\,NaH_2PO_4 \xrightarrow{heat} NaHPO_3\text{–}(OPO_2Na)_{n-2}\text{–}OPO_3HNa + (n-1)\,H_2O$$

**1** linear polyphosphate

$$n\,NaH_2PO_4 \xrightarrow{heat} n/x\,(NaPO_3)_x + n\,H_2O$$

metaphosphate



**2**

(trimetaphosphate: x = 3)

It is also known that upon degradation in aqueous solutions, polyphosphates with chain length $n \geq 5$ give rise, with the increase of *n*, to increasing amounts of trimetaphosphate (up to 60% of the total amount of phosphorus atoms, at pH 8 and 60°), see *Table 2* [3].

Table 2. *Yield of trimetaphosphate on heating aqueous solutions of linear polyphosphates 4–6 days at pH 8 and 60°* [3]

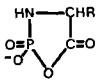
| Chain length *n* | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 20–100 |
|---|---|---|---|---|---|---|---|---|
| P as trimetaphosphate % | 0 | 0 | 0 | 5–8 | 15–17 | *ca.* 25 | *ca.* 38 | *ca.* 60 |

The availability of polyphosphates on the primitive earth has been discussed in several papers [4] [5]; their formation in prebiotic conditions is generally explained by the condensation (*e.g.* thermally catalysed or non catalysed dehydration) of hydrogenorthophosphates or by the reactions (for instance electric discharge reactions) of primitive atmospheres containing phosphine [6]. Phosphides (which upon reaction with water give rise to phosphine) are found in some meteorites [7], and the atmosphere of Jupiter, which is believed to be comparable to that of the primitive earth, contains small amounts of phosphine [8].

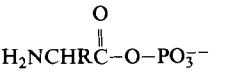
The central role that phosphorus derivatives (especially adenosinetriphosphate 'ATP' as energy carrier) play in the biochemical processes of contemporary living organisms, make it plausible that their prebiotic precursors during 'chemical evolution' might have been the cyclic or linear polyphosphates. This hypothesis is also supported by the fact that linear long chain polyphosphates are

still found today in some micro-organisms [9] and in some mammalian tissues [10]. If, in addition, we assume that present day biochemical pathways result from the evolution of prebiological reactions, our current knowledge enables us to conclude that the polyphosphates (linear of cyclic) still remain the most serious candidates for prebiotic condensation and phosphorylation reactions, especially in aqueous solutions [5].

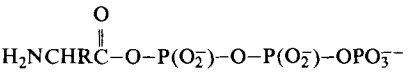
As described before [1], the peptide bond formation during the condensation of $\alpha$-amino acids in aqueous solution proceeds through the intermediate cyclic **3** or open **4** and **5** acylphosphates resulting from the reaction of the polyphosphate with one molecule of the amino acid; this intermediate acylates the amino group of a second molecule of the amino acid, thus yielding the peptide bond.



**3**
*N*-phosphonatoglycine cyclic anhydride



**4**
open amino-acylphosphate



**5**
aminoacyltriphosphate

This reaction proceeds well at pH 7–11 [1]. If a 0.1 M solution of glycine at pH 11.5–12.0 is made 0.3 M in sodium cyanide at the same pH, the yield of diglycine is almost doubled [11]; however, a pH of *ca.* 12 is rather improbable in prebiotic conditions. We were therefore interested to study the influence of the addition of sodium cyanide and other possible prebiotic 'condensing agents' or 'catalysts', on the polyphosphate and especially trimetaphosphate induced peptide synthesis in aqueous solutions at pH 8–9. Besides sodium cyanide and cyanamide we have chosen imidazole for the following reasons: a) imidazole and imidazole derivatives are produced in simulated prebiotic reactions [12], b) imidazole plays a catalytic role in acyl [13] and phosphoryl[2]) [14] transfer reactions (1-phosphonato-imidazole **6** and 1-triphosphonato-imidazole **7** transfer very easily the phosphoryl group to other sites and are often formed in model biochemical reactions as 'energy rich' intermediates [15]), and c) 1-($\alpha$-aminoacyl)imidazoles **8** give rise to peptides in aqueous solutions, the best yields of tripeptide (and higher peptides) being achieved precisely in the pH range 6–9 [16] which is of interest to us. Magnesium ion was also added in one experiment because of its catalysis of pyrophosphate breakdown in aqueous solutions [2], its catalysis of the synthesis of amino acid adenylate anhydride [17] and its use (together with imidazole) in simultaneous peptide and oligonucleotide formation in mixtures of amino acids and nucleoside-triphosphates [18]. We hoped therefore to obtain more significant amounts of tripeptide by adding imidazole (or imidazole and $MgCl_2$) to our aqueous system of polyphosphate and amino acid, and by working at pH 9 or below.

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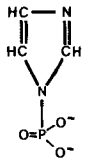
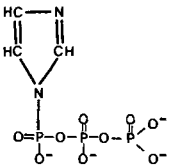
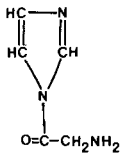
[2]) By phosphoryl transfer reactions, we mean the transfer of phosphorus containing groups *e.g.* $-P(O)O_2^{2-}$, $-P(O)O^--O-P(O)O_2^{2-}$, $-P(O)O^--O-P(O)O^--O-P(O)O_2^{2-}$, to molecules or sites containing acceptors such as alcohol, carboxyl or nitrogenous groups.



**6**
1-phosphonato-imidazole



**7**
1-triphosphonato-imidazole



**8**
1-glycylimidazole

All reactions were carried out in 0.1 M aqueous solutions of glycine at room temperature and at pH 8.1–9.0 either with sodium trimetaphosphate **2** or with sodium polyphosphate **1** ($n=26$). Then sodium cyanide, cyanamide, imidazole or imidazole/magnesium chlorid were added. After 14 days for the reactions with **2** and 26 days for the reactions with **1** ($n=26$), the solutions were analysed for their diglycine and triglycine content.

The results (see *Table 3*) indicate that in the presence of imidazole much higher yields of triglycine are obtained: with trimetaphosphate/imidazole the yield of triglycine is almost ten times higher (2.68%) than with trimetaphosphate alone (0.29%). The further addition of magnesium ions produces no appreciable change of the yields.

In contrast to what happened at pH 11.5–12.0 the addition of sodium cyanide to the trimetaphosphate/glycine solution at pH 8.3–9.0 diminishes the yields of diglycine and triglycine very significantly; the addition of cyanamide produces the same effect.

Table 3. *Diglycine and triglycine formation from 0.1 M aqueous solutions of glycine in the presence of trimetaphosphate or polyphosphate (n = 26) and sodium cyanide, cyanamide, imidazole or imidazole/$MgCl_2$, respectively, at room temperature and at pH 8.1–9.0*

| Polyphosphate | $c$[a]) | Other substance added | $c$[a]) | pH range[b]) | Reaction time days | Diglycine[c]) | | Triglycine[c]) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | $c \cdot 10^3$[a]) | % | $c \cdot 10^3$[a]) | % |
| – | – | Imidazole | 0.3 | 8.8–9.0 | 14 | – | – | – | – |
| Trimetaphosphate | 0.1 | – | – | 8.4–9.0 | 14 | 8.1 | 17.0 | 0.093 | 0.29 |
| Trimetaphosphate | 0.1 | Sodium cyanide | 0.3 | 8.3–9.0 | 14 | 3.6 | 7.5 | 0.007 | 0.02 |
| Trimetaphosphate | 0.1 | Cyanamide | 0.3 | 8.3–9.0 | 14 | 3.8 | 7.9 | 0.017 | 0.05 |
| Trimetaphosphate | 0.1 | Imidazole | 0.3 | 8.5–9.0 | 14 | 8.7 | 18.2 | 0.847 | 2.68 |
| Trimetaphosphate | 0.1 | Imidazole/$MgCl_2$ | 0.3/0.1 | 8.1–9.0 | 14 | 8.2 | 17.2 | 0.517 | 1.63 |
| Polyphosphate $n=26$ | 0.029 | – | – | 8.7–9.0 | 26 | 1.9 | 4.0 | traces | – |
| Polyphosphate $n=26$ | 0.029 | Sodium cyanide | 0.3 | 8.6–9.0 | 26 | 0.7 | 1.5 | traces | – |
| Polyphosphate $n=26$ | 0.029 | Cyanamide | 0.3 | 8.5–9.0 | 26 | 0.8 | 1.6 | traces | – |
| Polyphosphate $n=26$ | 0.029 | Imidazole | 0.3 | 8.5–9.0 | 26 | 2.2 | 4.6 | 0.067 | 0.20 |

[a]) $c$ in mol/l.

[b]) The pH is adjusted to 9.0 (pH meter) with concentrated ammonia every day; the left value is the lowest pH measured during the course of the reaction.

[c]) The yield is calculated relative to the initial glycine concentration, taking in account a dilution of 5% due to the daily corrections of the pH.

With sodium polyphosphate ($n=26$) the reaction of glycine is much slower in the chosen pH range (8.5–9.0) than with sodium trimetaphosphate: after 26 days the yields of diglycine and triglycine are only one fourth to one fifth of those obtained in the corresponding reactions with trimetaphosphate after 14 days, but the effect of the addition of sodium cyanide, cyanamide or imidazole is the same as in the case of trimetaphosphate.

Considering the reaction giving the best yields, *i.e.* the condensation of glycine in the presence of trimetaphosphate and imidazole at pH 8.3–9.0, we may admit that imidazole could react with trimetaphosphate to give 1-triphosphonato-imidazole **7** (and/or 1-phosphonato-imidazole **6**). Imidazole could also react with *N*-phosphonatoglycine cyclic anhydride **3** (resulting from the direct reaction of glycine with trimetaphosphate [1]) to give rise to 1-(*N*-phosphonatoglycyl)-imidazole or to 1-glycyl-imidazole **8** which is known to yield appreciable amounts of triglycine in aqueous solutions at pH 6.0–9.0 [16]; such possible intermediate 'energy-rich' derivatives which may also arise from other mechanisms could explain the catalytic role of imidazole in phosphoryl and acyl transfer reactions.

It is obvious that ammonia could also react with the proposed intermediates and yield products such as glycinamide, *etc.* Indeed, we have noticed on our chromatograms small peaks of ninhydrine-positive substances with long elution times (alkaline region), the most relatively important one corresponding to glycinamide. In order to avoid the formation of products derived from ammonia, the pH adjustments should be done with concentrated sodium hydroxide instead of concentrated ammonia.

The results obtained show that in the polyphosphate induced peptide synthesis in aqueous solutions the addition of imidazole promotes significantly the formation of tripeptides (and may be higher peptides) under conditions similar to those which might have prevailed on the primitive earth.

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## Experimental Part

1. *Starting materials.* Glycine, imidazole, cyanamide as well as the reference products diglycine and triglycine were commercial products (*Fluka AG,* Buchs SG). The samples of pure sodium trimetaphosphate and sodium polyphosphate with an average chain length of 26 were furnished by *Monsanto Chemical Company* (St. Louis, Missouri).

2. *Chromatographic analysis of amino acids and peptides.* Performed on a *Chromaspeak Rank Hilger* Amino Acid Analyser with an automatic integrator, pH gradient *ca.* 2.2–11.3. An aliquot of the sample was diluted 50 times with a pH 2.1 buffer and 150 µl of this solution were analysed using solutions of glycine, diglycine and triglycine as standards for the qualitative and quantitative analysis. Elution times in min: glycine 127.28, diglycine 186.51, triglycine 192.17, ammonia 311.76.

3. *Effect of the addition of sodium cyanide, cyanamide, imidazole or imidazole/$MgCl_2$, respectively, on the condensation of glycine in aqueous solutions of trimetaphosphate or polyphosphate* ($n=26$). Standard solutions of 0.612 g of sodium trimetaphosphate or 1.6 g of sodium polyphosphate ($n=26$) and 0.150 g of glycine in 20 ml of $H_2O$ were prepared. To these solutions were added 0.294 g of NaCN, 0.224 g of cyanamide, or 0.408 g of imidazole, respectively. To a trimetaphosphate/glycine

solution we also added in one experiment simultaneously 0.408 g of imidazole and 0.418 g of $MgCl_2 \cdot 6\,H_2O$. The pH of each solution was adjusted 9. A parallel experiment was carried out with a solution containing 0.150 g of glycine and 0.408 g of imidazole (it is already known that no condensation of glycine occurs with sodium cyanide [11] or cyanamide [5] at this pH). All these solutions were kept at RT., their pH measured every day and adjusted with conc. ammonia to 9 whenever necessary. During the reaction of glycine with trimetaphosphate and imidazole/magnesium chlorid, increasing amounts of ammonium magnesium phosphate precipitated. After 14 days for the reactions with trimetaphosphate and the reaction of glycine with imidazole, and after 26 days for the reactions with polyphosphate aliquots of each solution were analysed (see above). The results are given in *Table 3.* In all the chromatograms from the reactions with trimetaphosphate we noticed minor peaks in the alkaline region, the most relatively important one with an elution time of 275.78 min superimposing that of authentic glycinamide; the chromatograms from the reactions with polyphosphate, however, were not clear enough in that region to ascertain if those peaks were also present.

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