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Prebiotic Peptide-Formation in the Solid State

III. Condensation Reactions of Glycine in Solid State Mixtures Containing Inorganic Polyphosphates

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Summary. The reactions of glycine with inorganic polyphosphates in the solid state have been studied. The formation of peptides up to the decamer occurs at moderate temperatures (r.t.-100 °C) in the presence of imidazole and magnesium chloride. If adenosine 5'-monophosphate is added to the reaction mixture, 2'(3')-O-glycyl adenosine 5'-monophosphate is also obtained. These reactions could have occurred on the primitive earth.

Key words: Prebiotic Activation - Peptides - Inorganic Polyphosphates - Trimetaphosphate - Amino Acids

INTRODUCTION

If an aqueous solution containing glycine and an inorganic trimetaphosphate is adjusted to a slightly alkaline pH, glycylglycine and very small amounts of triglycine are formed (Feldmann, 1969; Rabinowitz, 1969; Rabinowitz et al., 1969). The mechanism of this condensation has been studied in detail, and it has been shown that the N-phosphates of glycylglycine and triglycine are intermediates (Chung et al., 1971). However, the prebiotic significance of the reaction is unclear, since it occurs only in alkaline solution and yields very small amounts of peptides other than glycylglycine.

In the present paper we discuss reactions of glycine and condensed inorganic phosphates in the solid state. We show that in the presence of imidazole and Mg^{2+}, oligoglycines,

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Table 1. Chromatographic and electrophoretic mobilities of compounds (gly = 1.00)

	I R_f	II R_f	III R_m	IV R_m
Glycine	1.00		1.00	1.00
$(gly)_2$	1.00		2.04	6.95
$(gly)_3$			1.94	
$(gly)_4$			1.74	
$(gly)_5$			1.61	
$(gly)_6$			1.49	
$(gly)_7$			1.43	
$(gly)_8$			1.34	
$(gly)_9$			1.30	
$(gly)_{10}$			1.24	
DKP	1.84		0.23	1.00
N-formyl glycine	2.22		-0.43	20.0
pA	0.45		0.20	19.0
pA-gly	0.47		1.56	
P_1		1.00[a]		
P_2		0.51[a]		
P_3		0.27[a]		
$P_3!$		0.90[a]		

[a] $P_1 = 1.00$

at least up to the decamer, can be obtained in appreciable yield. Furthermore, in the presence of adenosine 5'-phosphate, small yields of 2'(3')-O-glycyl adenosine 5'-phosphate are formed. Thus condensed inorganic phosphates can replace organic phosphates such as ATP, AppA or ImpA in the activation of glycine in the solid state (Lohrmann et al., submitted for publication).

EXPERIMENTAL

a) Materials and Analytical Methods[1]

Sodium trimetaphosphate, sodium tripolyphosphate and sodium hexametaphosphate were purchased from Monsanto Chemicals.

[1] Abbreviations: pA, adenosine 5'-monophosphate; pA-gly, 2'(3')-O-glycyl-adenosine 5'-monophosphate; $(gly)_n$, oligoglycine; DKP, diketopiperazine; P_1, orthophosphate; P_2, pyrophosphate; P_3, tripolyphosphate; $P_3!$,trimetaphosphate; AppA, P_1,P_2-diadenosine 5'-pyrophosphate; ImpA, adenosine 5'-phosphorimidazolide.

Glycine was purchased from Calbiochem. Adenosine 5'-monophosphate and imidazole were from Sigma Chemicals. Ammonium chloride and magnesium chloride (Mallinckrodt) were of analytical grade. Radioactive ^{14}C-glycine was obtained from Schwarz and purified by electrophoresis in the system III.

Paper chromatography was carried out by the descending technique using Whatman 3MM paper in the following systems: I, n-butanol and 5N acetic acid (2:1); II, 95% ethanol and 1M ammonium acetate (7:3). Electrophoresis was carried out on Whatman 3MM paper in the following systems: III, 0.05M formic acid buffer adjusted to pH 2.7 with ammonia; IV, 0.03M potassium phosphate, pH 7.1. R_f and R_m values of the compounds are listed in Table 1.

The chromatograms were passed through a Baird Atomic RSC-363 scanner with integrator. The yields are determined as the percentage of the total radioactivity on the paper, after correcting for background.

The identification of oligoglycines with chain length up to five and diketopiperazine was carried out by comparing their mobilities with those of authentic samples obtained commercially. Oligoglycines separated well on electrophoresis in system III. In some experiments, several radioactive products moving more slowly than pentaglycine were detected as shown in Fig.1. We believe that they are hexaglycine, heptaglycine, octaglycine, nonaglycine and decaglycine, but standards were not available. pA-gly was identified by co-chromatography with an authentic sample prepared from N-t-BOC-glycine and pA according to Gottikh et al. (1970). Its identity was confirmed by base hydrolysis of the eluate to glycine and pA. N-Formylglycine was identified by co-chromatography with an authentic sample prepared from glycine and formic acid (Greenstein & Winitz, 1961). Inorganic phosphates were identified by comparing the chromatographic mobilities with those of authentic samples in system II. Phosphates were detected using the Hanes-Isherwood procedure (H. Rosenberg, 1969).

b) Solid State Reaction

General Procedure. A solution containing ^{14}C-labelled glycine (0.033M, 0.125 mc/mmole), imidazole (0.33M), $MgCl_2$ (0.033M), sodium trimetaphosphate (0.033M), was titrated to the required pH with HCl. The solution was put on squares of Whatman GF 82 glass-fiber paper with 0.1 ml of the solution on each 1 x 1 cm square. The squares were dried over P_2O_5, under vacuum, at room temperature for 24 hrs and heated

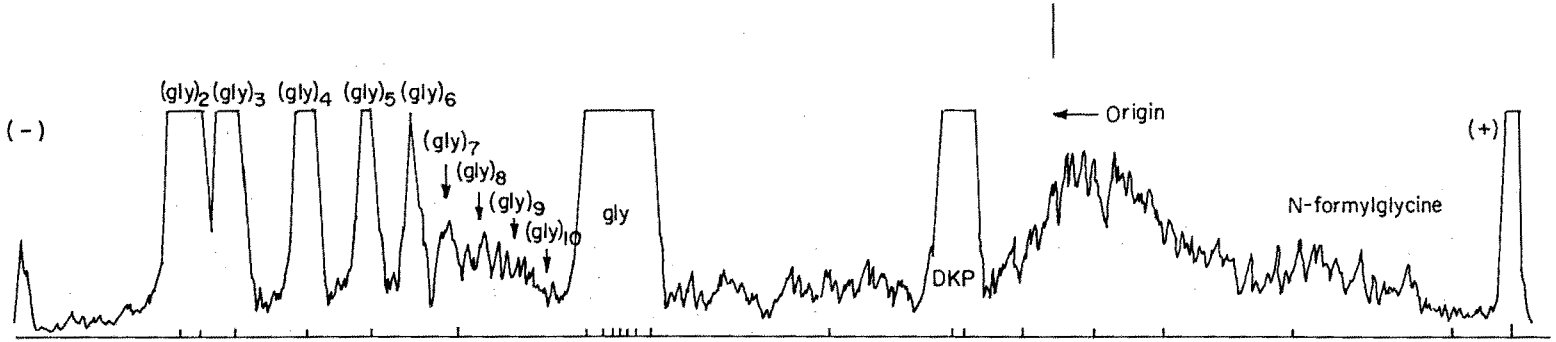


Fig.1. Radioscan of the electrophoretogram in system III of the products from 4 hrs-reaction of glycine with trimetaphosphate in the presence of imidazole and magnesium chloride at pH 6.0 and 100 $^{\circ}$ in the solid state

at the required temperatures in open test tubes with exposure to laboratory humidity. After the reaction, samples were withdrawn and eluted with 0.2 ml of 0.05M formic acid, and analyzed. Control experiments in which either imidazole or $MgCl_2$ was omitted were carried out in an identical manner.

The following reactions were carried out in the same way:

(i) Reactions in which linear tripolyphosphate (0.033M) or commercial hexametaphosphate (0.033M) was used instead of trimetaphosphate;

(ii) Reactions at pH 8 in which 0.33M of NH_4Cl was added to the standard reaction mixture;

(iii) Reactions in which the initial solution contained ^{14}C-glycine (0.033M, 0.125 mc/mmole), sodium trimetaphosphate (0.033M), pA (0.033M), $MgCl_2$ (0.033M) and imidazole (0.33M), either without NH_4Cl at pH 6.0 or with NH_4Cl (0.33M) at pH 8.0. The thermal reactions of dried samples were carried out either with exposure to laboratory humidity, or in a dry atmosphere.

c) Acidity of Reaction Mixtures after Heating

Reaction mixtures (1.0 ml) containing glycine (0.033M), sodium trimetaphosphate (0.033M), $MgCl_2$ (0.033M), imidazole (0.33M) and NH_4Cl (0.33M) were dried and heated in glass tubes. Then water was added to the dried sample to make up a total volume of 1.0 ml. The pH value of the resulting solution was measured with a Beckman pH meter.

RESULTS

a) Polypeptide Formation in Solids Derived from Solutions Adjusted to pH 6

Glycine condenses efficiently to a series of oligoglycines at least up to the decamer when it is heated with equimolar amounts of Mg^{2+}, imidazole and inorganic trimetaphosphate. Results obtained at different temperatures and with mixtures of various compositions are collected in Table 2. The electrophoresis pattern obtained in the system III from one of our more efficient reactions is illustrated in Fig.1. We always obtained substantial amounts of diketopiperazine in these experiments and small amounts of N-formylglycine. In addition, variable amounts of unidentified decomposition products that remained close to the origin on electrophoresis of the system III were always detected. When larger amounts

Table 2

Peptides formation from glycine with trimetaphosphate in the presence of imidazole and magnesium chloride (gly = 0.033M; molar ratio, gly:P_3! : Im : $MgCl_2$ = 1:1:10:1)

Temp. (°C)	Time (hr)	$\frac{P_3!}{gly}$	Yield (%) glycine	$(gly)_2$	$(gly)_3$	$(gly)_4$	$(gly)_5$	$(gly)_6$	$(gly)_7$	$(gly)_8$	$(gly)_9$	$(gly)_{10}$	DKP	N-formyl glyc.	un-ident. prod.
r.t.	120	1	87.2	3.7	0.9								2.1	1.7	4.2
65	8	1	78.8	6.9	1.7	0.9	0.4	0.2					4.9	1.2	4.9
	24	1	72.4	8.9	2.4	1.5	0.9	0.5					5.5	1.3	6.6
	48	1	72.1	8.6	3.5	1.8	1.2	0.6	1.0[a]				5.7	1.2	4.4
	240	1	61.7	8.0	4.2	2.3	1.5	1.0	0.6	1.1[a]			7.7	0.9	11.0
85	8	1	54.8	12.2	5.1	3.4	2.0	1.0	0.8	0.5	0.3	0.7[a]	8.6	1.4	9.2
100	1	1	63.3	12.9	3.6	1.9	0.8	0.4	0.2[a]				8.1	1.6	7.2
	2	1	53.8	13.4	5.4	3.0	1.4	1.0	0.6	0.3[a]			9.5	1.8	9.8
	4	1	39.9	11.1	7.0	5.2	3.7	2.4	1.4	1.2	0.7	1.0[a]	12.8	1.2	12.4
65	24	2	67.0	7.7	3.6	1.7	0.8	0.4					4.0	2.8	12.0
85	8	2	44.9	6.7	4.0	3.5	2.4	1.2	0.6	0.6[a]			10.9	2.2	23.1
100	6	2	21.9	3.1	3.3	3.1	2.7	2.1	1.4	1.1	0.7	1.4[a]	22.1	1.4	35.6
65	24	5	62.0	9.4	3.1	1.7	0.7	0.4					6.6	3.6	12.6
85	8	5	30.7	4.3	2.6	2.1	1.9	1.3	0.6	1.3[a]			17.3	2.0	35.8
100	6	5	12.2	1.0	1.4	2.2	1.9	1.4	1.0	0.7	0.4	0.9[a]	37.1	1.6	38.2

[a] Higher peptides than indicated are included.

Table 3

Reaction of glycine with trimetaphosphate in the absence of imidazole and magnesium chloride (gly = 0.033M; molar ratio, gly : P_3! : Im : $MgCl_2$ = 1:1:10:1)

Temp. (°C)	Time (hr)	pH	$MgCl_2$	Im	Yield (%) glycine	$(gly)_2$	$(gly)_3$	$(gly)_4$	$(gly)_5$	DKP	N-formyl glycine	unidentified products
65	24	6.0	+	-	100							
85	8		+	-	100					trace		
100	4		+	-	98.7	0.5				0.6		
65	24	6.0	-	+	96.3	trace				1.8	0.6	1.2
85	8		-	+	92.6	"				2.7	0.5	4.1
100	4		-	+	90.4	1.3	0.2	0.1		3.8	0.5	3.6
100	4	8.0	-	+	60.5	0.4	0.4	0.8	0.5	15.5	13.3	8.6
100	4	8.0	+	-	96.8	0.7				1.3	0.2	1.0

Table 4

Reaction of glycine with tripolyphosphate and hexametaphosphate in the presence of imidazole and magnesium chloride at pH 6.0 (glycine = 0.033M; molar ratio, gly : Pn : $MgCl_2$: Im = 1:1:1:10)

Temp. (°C)	Time (hr)	Yield (%) glycine	$(gly)_2$	$(gly)_3$	$(gly)_4$	$(gly)_5$	$(gly)_6$	$(gly)_7$	$(gly)_8$	$(gly)_9$	DKP	N-formyl glycine	unidentified products	
65	24	98.2	0.4								1.3			a
85	8	94.6	0.6								1.8		2.8	a
100	4	78.4	5.8	1.9	0.7	0.4					11.0	0.8	1.0	a
65	24	82.5	7.1	1.6	0.6	0.3					3.8	0.6	3.5	b
85	8	56.3	7.3	5.0	4.7	2.1	1.0	0.4	1.0[c]		14.1	1.0	7.1	b
100	4	29.9	4.8	4.2	3.9	4.0	3.2	2.2	1.8	2.6[c]	23.9	1.1	18.4	b

[a]Tripolyphosphate was used. [b]Hexametaphosphate was used. [c]Higher peptides than indicated are included.

Table 5

Reaction of glycine with trimetaphosphate in the presence of imidazole and magnesium chloride at pH 8.0 (glycine = 0.033M; molar ratio, gly : P_3! : $MgCl_2$: Im = 1:1:1:10)

Temp. (°C)	Time (hr)	$\frac{NH_4Cl}{gly}$	Yield (%) glycine	$(gly)_2$	$(gly)_3$	$(gly)_4$	$(gly)_5$	$(gly)_6$	$(gly)_7$	$(gly)_8$	$(gly)_9$	$(gly)_{10}$	DKP	N-formyl glyc.	un-identified products
65	24	0	44.0	8.6	4.7	3.0	2.2	2.0	1.0	0.7	0.9[a]		4.6	2.0	26.4
85	8	0	32.8	5.2	2.5	2.2	1.7	1.3	1.0	0.9	0.7	1.0[a]	13.6	2.4	34.6
100	4	0	16.5	0.7	0.6	0.7	0.7	0.7	0.7	0.7	0.5	1.2[a]	17.1	6.8	53.1
65	24	10	69.2	9.8	3.4	2.0	0.9	0.3	0.3	0.1			5.6	1.4	7.3
85	8	10	45.5	13.1	7.1	4.8	3.5	1.4	1.0	0.6	0.6[a]		11.3	0.6	10.4
100	4	10	47.3	9.2	5.9	4.7	3.4	2.2	1.1	0.7	0.3	0.7[a]	14.1	0.6	9.7

[a] Higher peptides than indicated are included.

Table 6

Reaction of glycine with Trimetaphosphate and adenosine 5'-monophosphate in the presence of imidazole and magnesium chloride (glycine = 0.033M; molar ratio, gly : P_3! : pA : Im : $MgCl_2$ = 1:1:1:10:1)

Temp. (°C)	Time (hr)	pH	$\frac{NH_4Cl}{gly}$	glycine	$(gly)_2$	$(gly)_3$	$(gly)_4$	$(gly)_5$	Yield (%) $(gly)_6$	pA-gly	DKP	N-formyl-glycine	unidentified products	
65	24	6.0	0	86.0	5.5	0.5				0.5	2.7	2.2	2.7	
85	8			73.4	8.9	2.0	0.6	0.3		0.5	6.5	2.6	5.3	
	8			64.9	11.9	2.6	0.6	0.4	0.2	2.5	8.4	2.6	5.8	a
100	4			60.7	11.8	3.9	1.5	0.7	trace	1.4	11.4	1.0	7.5	
	4			60.7	11.6	3.4	1.2	0.7	0.3	1.7	12.4	1.9	6.2	a
65	24	8.0	10	94.1	2.8	0.3	trace			0.3	0.5	1.2	0.8	
85	8			87.1	6.2	1.0	trace			0.3	2.2	1.3	1.9	
	8			82.7	7.6	1.1	0.4	0.2		1.2	3.4	1.3	2.1	a
100	4			66.6	11.3	2.4	0.7	0.3		0.5	11.2	2.0	4.9	
	4			60.9	13.3	4.2	0.8			1.5	11.6	1.5	6.2	a

[a]Thermal reaction was carried out in a dry atmosphere.

of trimetaphosphate were added to the reaction mixtures, the main effect was to increase the yield of decomposition products.

It is striking that in this system some diglycine and diketopiperazine are obtained even at room temperature. At 65 °C the total yield of peptides and diketopiperazine was in excess of 25% after 10 days, and included 1.1% of materials higher than the heptamer. At 100 °C the yield of condensed materials exceeded 45% and included 1% of material higher than the nonamer.

If imidazole is omitted from the reaction mixture, the glycine remains almost unchanged even at 100 °C (Table 3). If Mg^{2+} is omitted from the reaction mixture the rate of reaction is greatly reduced, but small amounts of peptides are formed at 100 °C after 4 hours (Table 3).

If glycine is heated with Mg^{2+}, imidazole and linear tripolyphosphate, the yield of peptides is much smaller than with trimetaphosphate under the same conditions (Table 4). On the other hand, when a long chain polyphosphate (commercial hexametaphosphate) is substituted for trimetaphosphate, the yield of peptides is only moderately reduced (Table 4).

b) Polypeptide Formation in Solids Derived from Solutions at pH 8

If a solution containing glycine, imidazole, Mg^{2+} and trimetaphosphate is adjusted to pH 8 before evaporation, peptides are formed more rapidly than from solutions at pH 6. However, the peptides are less stable, and large amounts of unidentified decomposition products are obtained (Table 5). If NH_4Cl is added to the original solution, decomposition is largely suppressed and excellent yields of peptides are obtained (Table 5).

The effect of NH_4Cl is due to the acidity developed in the solid reaction mixtures through the loss of ammonia. When the pH's of the solutions formed by redissolving the reaction mixtures were measured, we found that if the initial pH was 8.05, the pH after drying at room temperature was 6.75. Further heating at 65 ° (24 hrs) or 100 ° (2 hrs) reduced the pH to 6.34 or 6.05, respectively. There was little change in pH on heating if NH_4Cl was omitted from the reaction mixture.

The omission of imidazole from the reaction mixture reduces the yield of peptides to a very low value, even at 100 °C. The omission of Mg^{2+} from the reaction mixture reduces the rate of peptide-formation very substantially, but permits the synthesis of substantial amounts of diketopiperazine and N-formylglycine (Table 3).

c) The Formation of pA-gly

If pA is added to the standard reaction mixtures, the yield of peptides is reduced substantially. However, small amounts of pA-gly are formed. The yields never exceed 2-3%; they are larger in more acidic mixtures and smaller in mixtures obtained from solutions at pH 8 (Table 6).

The effect of humidity is particularly noteworthy. While the yields of peptides are little affected by changes in humidity, pA-gly is obtained in better yield under dry conditions. This confirms our previous observation that the 2'(3')-glycyl esters of nucleotides, unlike peptides, are very sensitive to hydrolysis by atmospheric moisture.

d) Analysis of the Inorganic Decomposition Phosphates Formed from $P_3!$ in the Condensed Reaction

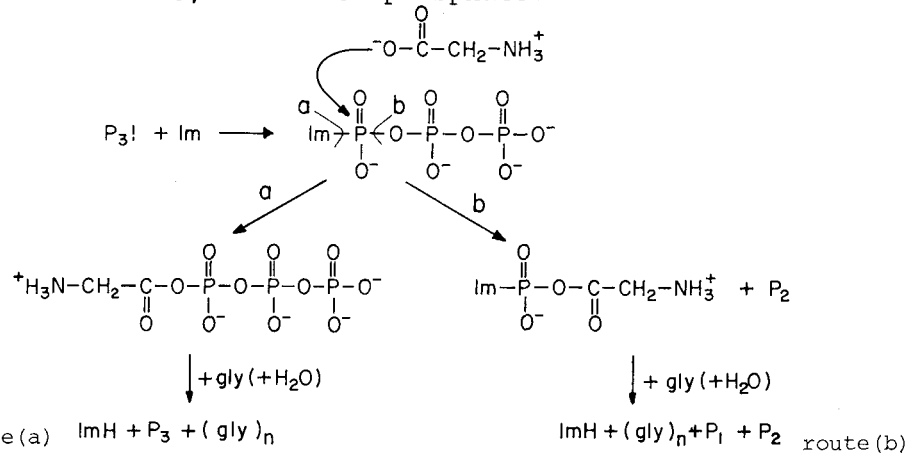
At 65 °C almost all of the trimetaphosphate that decomposes is converted to linear tripolyphosphate. At higher temperatures some pyrophosphate and orthophosphate are formed, perhaps by hydrolysis of linear tripolyphosphate.

DISCUSSION

a) Reaction Mechanisms

It is difficult to deduce mechanisms from data presented in this paper, since the efficiency of solid-state reactions depends so much on the way in which the phases deposit from solution. The following conclusions are, therefore, extremely tentative.

Analogy with the system discussed in a previous paper (Chung et al., 1971) suggests that the first step in the reaction is the attack of imidazole to trimetaphosphate to give a reactive, linear triphosphate:



This intermediate could then react with the carboxylate group of the amino acid according to a or b to give an activated derivative capable of forming peptide bonds. Since the major hydrolysis product is linear tripolyphosphate, under conditions in which peptide formation is extensive, we believe that route (a) is indicated. However, we emphasize that this mechanism is not proven, and various others could be proposed, for example, one involving a primary attack of glycine on trimetaphosphate.

b) Prebiotic Significance

We have shown that linear polyphosphates are formed when inorganic phosphates are heated in the solid-state with urea and ammonium chloride (Osterberg& Orgel, 1972). The hydrolysis of long-chain linear polyphosphates generates trimetaphosphate in high yield (Thilo et al., 1953). These two reactions together could have led to the synthesis of large quantities of linear long-chain polyphosphates and trimetaphosphate on the primitive earth. Alternatively, trimetaphosphate could have formed directly from inorganic phosphate, ammonium chloride and urea in the presence of a nucleoside (Osterberg & Orgel, 1972).

In earlier papers of this series we have shown that condensed organic phosphates, such as ATP or AppA may be used to activate amino acids in the solid state. The amino acids then condense to form peptides or react with nucleotides to form 2'(3')-esters (Sawai et al., submitted for publication). Our new results extend this work and show that linear polyphosphates or trimetaphosphate could equally well have provided the energy needed for the prebiotic activation of amino acids.

The relationship of our findings to other work on solid-state peptide synthesis and to protein synthesis in living systems has already been discussed in detail (Lohrmann & Orgel, 1973; Sawai et al., submitted for publication).

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