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THE MECHANISM OF THE TRIMETAPHOSPHATE-INDUCED PEPTIDE SYNTHESIS

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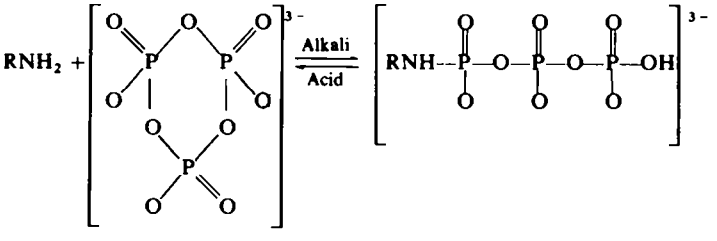
Abstract—The formation of peptides from glycine in the presence of trimetaphosphate proceeds mainly via peptide-N-phosphates as intermediates. The interaction of glycine with trimetaphosphate leads first to the formation of a cyclic acylphosphoamidate and pyrophosphate. The cyclic compound then reacts with the free amine group of glycine or diglycine to give diglycine-N-phosphate or triglycine-N-phosphate.

INORGANIC trimetaphosphates, in alkaline aqueous solution, bring about the condensation of amino acids such as glycine to dipeptides.[1] The direct activation of the carboxyl group under these conditions seems unlikely since simple carboxylic acids react very slowly with trimetaphosphate.[2] On the other hand, Feldman[3] has shown that ammonia and alkylamines react to form open chain phosphoramidates which revert to amines and trimetaphosphate in acid solution. Here we describe the intermediates in the peptide synthesis reaction.



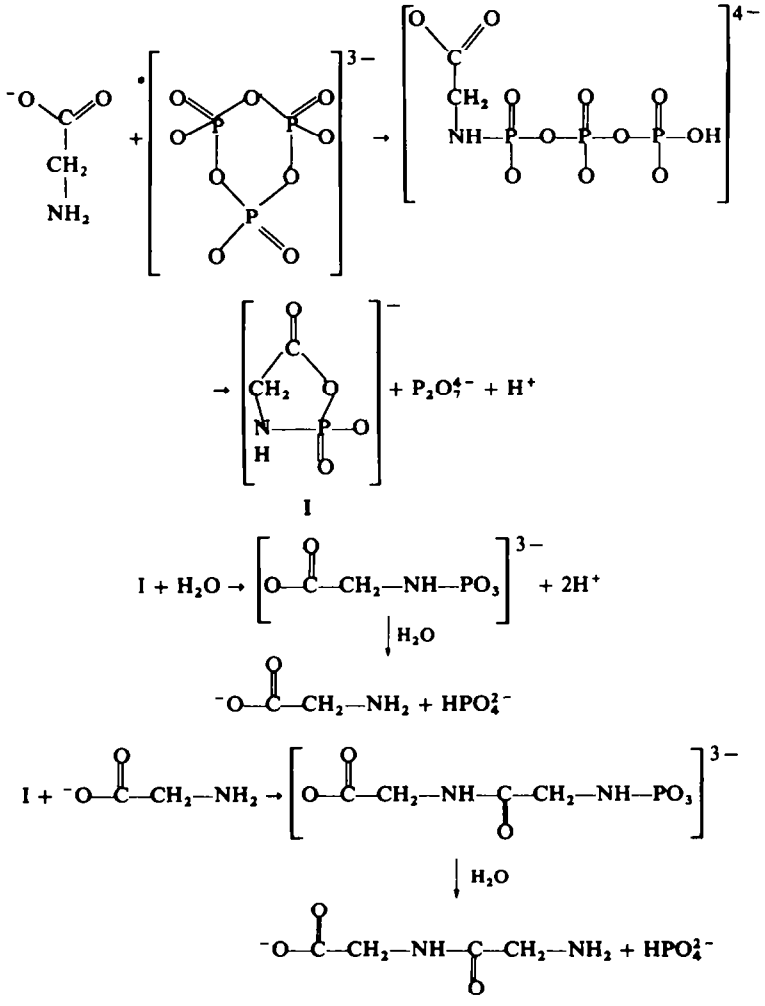
Solutions 0·1 M in glycine and 0·1 M in trimetaphosphate, initially adjusted to pH's 8, 10, 12 with sodium hydroxide, were allowed to react for 10 days. The yield of diglycine obtained on electrophoresis in the highly acidic System E was negligible from the reaction at pH 8, and amounted to 23% and 28% from reactions at pH's of 10 and 12, respectively. Comparison of experiments in which tetramethylammonium or sodium hydroxide were used for alkaline titrations (Table 1) suggest that the reaction is catalyzed by sodium ions.

When the same experiment was analyzed in System D, glycine-N-phosphate,

diglycine-N-phosphate and triglycine-N-phosphate were detected in addition to diglycine and a very small amount of triglycine. In Fig 1 we show the yields of products as a function of time. Clearly there is a rapid initial formation of glycine-N-phosphate and diglycine-N-phosphate. Then diglycine arises by hydrolysis of its N-phosphate. Thus, much of the diglycine detected by Rabinowitz[1] must have arisen from the hydrolysis of diglycine-N-phosphate.

The reaction of diglycine with trimetaphosphate is quite different. The sole product is diglycine N-triphosphate which reverts in acid to diglycine and trimetaphosphate. Clearly diglycine behaves as a simple amine in this reaction; the carboxyl group does not participate under these conditions.

A simple explanation of all the observations on peptide synthesis is provided by the following scheme



Glycine first attacks the cyclic triphosphate in the usual way to form an open chain phosphoramidate. However, the neighbouring carboxyl group now expels the pyrophosphate anion with the formation of a cyclic acylphosphoramidate. This sensitive acyl-phosphate is then attacked either by water or by a second glycine molecule. The pH dependence of the yield of peptide is explained by the unreactive nature of the $H_3\overset{+}{N}\text{-}CH_2CO_2^-$ zwitterion formed at lower pH's ($pK_2 = 9{\cdot}60$).

A number of related reactions are known, for example the phosphorylation of cis-glycols by trimetaphosphate via cyclic phosphates.[4] Closely related 5-membered cyclic intermediates have been postulated in a number of reactions of phosphate esters which contain an α-carboxylic acid group.[5]

The products from the reaction of a tenfold excess trimetaphosphate with mixtures of radioactive diglycine and cold glycine in different proportions (Table 2) are consistent with the above scheme. All of the glycine is converted to phosphates. Two radioactive substances are obtained, diglycine-N-triphosphate formed by direct reaction of diglycine and trimetaphosphate and triglycine-N-phosphate formed by the reaction of diglycine with the cyclic acylphosphoramidate intermediate from cold glycine.

There are a number of reasons for doubting the prebiotic significance of these reactions. It is not easy to understand the prebiotic origin of trimetaphosphate. Furthermore, the protection of the amino group by phosphorylation prevents the formation of high oligopeptides. Nonetheless, a very slow accumulation of oligopeptide by this method might be possible if trimetaphosphate were supplied in large amounts over a long period of time.

EXPERIMENTAL

Materials and methods. Glycine was purchased from General Biochemicals, diglycine from Nutritional Biochemicals Corporation, triglycine and tetraglycine from Mann Research Laboratories. Radioactive

TABLE 1. PERCENTAGE YIELD OF DIGLYCINE FROM THE REACTION OF GLYCINE AND INORGANIC TRIMETAPHOSPHATES AT ROOM TEMPERATURE[a]

Initial pH	pH (after 4 days)	Cation	Days 1	Days 4
8	7·6	Na	0	0[b]
10	9·15	Na	13	23
12	9·6	Na	18	28[c]
10	9·2	NH_4	11	18
12	9·9	$N(CH_3)_4$[d]	1·5	7
12	9·65	$N(CH_3)_4$[e]	15	24

[a] Analyses were carried out in highly acidic system E. All N-phosphates were hydrolyzed to glycine and diglycine.

[b] In additional experiments the pH was adjusted back to 8 occasionally. No dimer was observed.

[c] A small amount of triglycine was obtained.

[d] $N(CH_3)_4OH$ was used to adjust the pH value.

[e] NaOH was used to adjust the pH value.

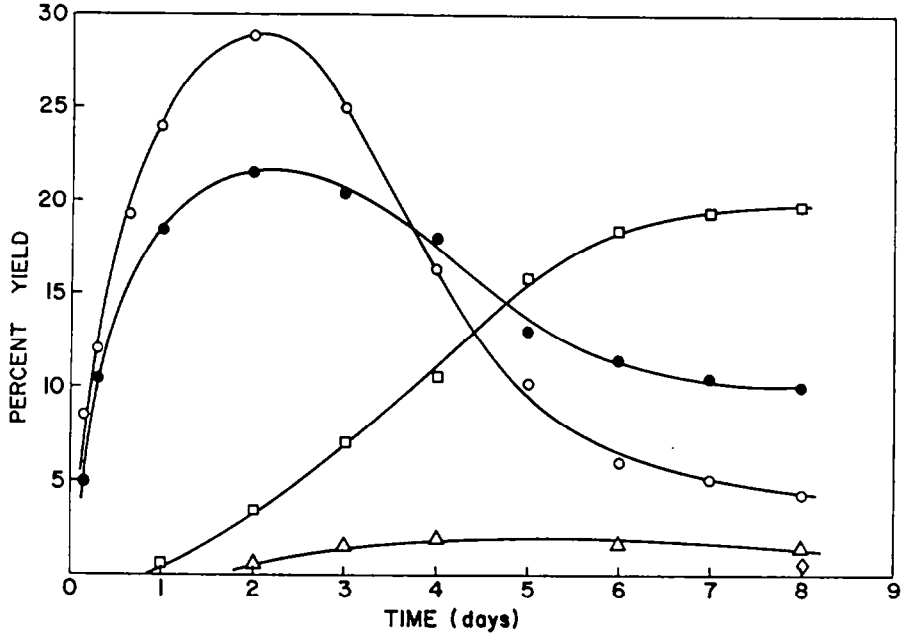


FIG 1. The yield of glycine-N-phosphate, diglycine-N-phosphate, triglycine-N-phosphate, diglycine and triglycine as a function of time in the reaction of glycine (0·1 M) with sodium trimetaphosphate (0·1 M) under alkaline conditions (initial pH 12).
—○—○— Glycine-N-phosphate —●—●— Diglycine-N-phosphate —□—□— Diglycine —△—△— Triglycine-N-phosphate ◇ Triglycine

TABLE 2. PERCENTAGE OF TRIGLYCINE*-N-PHOSPHATE AND DIGLYCINE*-N-TRIPHOSPHATE FROM THE REACTION OF GLYCINE AND DIGLYCINE* WITH 1 M TRIMETAPHOSPHATE AT ROOM TEMPERATURE[a, b]

No.	Glycine (Mole)	Diglycine* (Mole)	Diglycine*-N-Triphosphate	Triglycine*-N-phosphate
1	0·01	0·09	90·5	9·5
2	0·05	0·05	74	26
3	0·09	0·01	56	44

[a] An asterisk is used to designate radioactive compounds.
[b] pH value of the reactions was 12.

glycine was purchased from Schwarz Bioresearch and radioactive diglycine from International Chemical and Nuclear Corporation. The specific activity was 0·1 μC per μmole of glycine in each case. Sodium trimetaphosphate was obtained as a research sample from Monsanto Chemical Company. Tetramethyl ammonium trimetaphosphate and the ammonium salt were obtained by a procedure described by Saffhill.[4]

The Mg salts of glycine-N-phosphate, diglycine-N-phosphate were synthesized by a published procedure.[6] The Mg salt of triglycine-N-phosphate was synthesized in a similar way. Its identity was established by hydrolysis to triglycine and inorganic orthophosphate. Na salts of these compounds were obtained from the Mg salts using a Dowex-50 cation exchange resin, Na ion form.

TABLE 3. R_f VALUES AND MOBILITIES

	A	B	C	D	E
Glycine	0·61				0·78[c]
Diglycine	0·61				1·22[c]
Triglycine	0·59				1·05[c]
Glycine-N-phosphate	0·36		0·63	1·82[b]	
Diglycine-N-phosphate	0·43		0·66	1·64[b]	
Triglycine-N-phosphate	0·43		0·77	1·43[b]	
Diglycine-N-tripolyphosphate	0·32		0·93		
Orthophosphate		0·90	0·71		
Pyrophosphate		0·73	0·22		
Trimetaphosphate		0·49	0·19		
Tripolyphosphate		0·33	0·40		

[a] R_f values are very sensitive to the ratio of dioxan and water used.

[b] Referred to mobility of adenylic acid as unity.

[c] Referred to mobility of adenosine as unity. The mobility of tetraglycine is 0·98.

Chromatography was carried out by the descending technique in the following systems: System A, n-propanol, conc ammonia, water (55:10:35 v/v); System B, dioxan, water, conc ammonia, trichloroacetic acid, (80 ml: 25 ml: 0·5 ml: 2·5 g); System C, 0·1 M NaCl. Whatman 3 MM paper was used for System A, Whatman 1 paper for System B and Whatman AE 81 paper (aminoethyl cellulose paper) for System C. Electrophoresis was carried out on Whatman 3 MM paper in 0·03 M potassium phosphate (pH 7·2) (System D) and in 0·05 M formate (pH 2·7) (System E). R_f values and mobilities are collected in Table 3.

Chromatograms of labelled compounds were cut into strips and run through a Baird-Atomic RSC-363 scanner with integrator. The radioactive zones were cut out and counted more accurately in a Beckman LS-200 liquid scintillation counter. Papers were put directly in liquifluor diluted with toluene.

After chromatography, non-radioactive amino acid derivatives could be detected either with a ninhydrin spray or with a phosphate spray. In the ninhydrin test, chromatograms were first sprayed with ninhydrin soln (0·3% ninhydrin in sat n-BuOH soln) and then heated to 100° in an oven for 5 min. Glycine and peptide gave purple and yellow colored spots, respectively. All N-phosphates also gave a positive ninhydrin test under these conditions, presumably due to the hydrolysis at 100° by the BuOH-water mixture. In the phosphate test, the chromatogram was first sprayed with Hanes-Isherwood solution[7] and heated in an oven at 85° to generate the phosphomolybdate complex. The latter was then converted to molybdenum blue by reduction with stannous chloride solution.[7]

Amino acid N-phosphates were usually eluted from paper with water. However, when system C was used, elution was carried out with 0·05 M triethylammonium bicarbonate soln (pH = 8·7). The eluate was evaporated to a small volume and 2·5 N AcOH added. The mixture was left at room temp for 30 min. It was, then, divided into two parts. One was run in System E to separate peptides. The other was run in System B to separate inorganic phosphates.

The identification of glycine-N-phosphate, diglycine-N-phosphate and triglycine-N-phosphate as products was confirmed by clean hydrolysis to amino acid or peptide and inorganic orthophosphate as well as by coincidence with authentic samples in all relevant chromatography and electrophoresis systems. The structure of diglycine-N-triphosphate was demonstrated by clean hydrolysis to diglycine and trimetaphosphate in acid solution.

After acid hydrolysis, some typical reaction mixtures were analyzed on an amino acid analyser. These analyses confirmed the results obtained by electrophoresis in System E.

General procedures. Solns of glycine or diglycine and trimetaphosphate at appropriate concentrations were mixed together at room temp. pH values were adjusted with 50% NaOH or by other bases at the beginning of the reaction as indicated in Table 1. Glycine and peptides were separated in System E. The N-phosphates were separated in Systems A or D. Eluted samples were hydrolyzed as described above.

When excess trimetaphosphate was used, inorganic phosphates were first separated from the N-phosphates in System C.

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