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158. Peptide and Amide Bond Formation in Aqueous Solutions of Cyclic or Linear Polyphosphates as a Possible Prebiotic Process

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(1. VI. 70)

Résumé. En faisant réagir des acides aminés en solution aqueuse, à température ordinaire et à pH env. 11, en présence de polyphosphates cycliques ou linéaires, on obtient des dipeptides avec des rendements allant jusqu'a 40%. Dans le cas de la sérine, on note uniquement la formation du dérivé O-phosphorylé, soit l'acide sérinephosphorique.

En faisant réagir un mélange de glycine (ou d'alanine) et de l'une des trois amines suivantes: phosphosérine, phosphoéthanolamine ou acide amino-2-éthanephosphonique, en solution aqueuse en présence de trimétaphosphate, on obtient à côté de la diglycine des quantités appréciables d'amide mixte, soit la N-glycyl-phosphosérine, la N-glycyl-phosphoéthanolamine et l'acide N-glycyl-amino-2-éthanephosphonique respectivement. Nous pensons que des peptides, phosphopeptides et amides mixtes de ce type sont également formés lors de réactions par décharge électrique dans des systèmes contenant de la phosphine, du méthane, de l'ammoniac et de l'eau.

Un mécanisme probable conduisant à la liaison peptidique ou amide serait la formation intermédiare d'un acylphosphate cyclique ou ouvert de l'acide aminé, qui réagirait ensuite avec le groupe amino d'une deuxième molécule d'acide aminé (→ peptide) ou de l'amine (→ amide).

Ces réactions présenteraient un certain intérêt dans la formation prébiotique de la liaison peptidique et amide, ainsi que de la liaison ester phosphorique.

We have reported that several amino acids are condensed to dipeptides in aqueous solutions of cyclic or linear polyphosphates, at pH above 7, at 70° and room temperature [1]. In the case of cyclic polyphosphates the best yields are obtained with trimetaphosphate, while for linear polyphosphates the yields increase with their average chain lengths. When hydroxy amino acids like serine are used, the main product from the reaction is O-phosphoserine, and only very small amounts of peptide are formed [1].

We have also previously found that when electric discharges are passed through mixtures containing phosphine[1]), methane, ammonia, and water (believed to be

[1]) The possibility of existence of phosphine on the primitive earth has already been discussed [2].

constituents of primitive atmospheres), there is evidence of the formation of phosphorus-containing amines including O-phosphoethanolamine, O-phosphoserine, and 2-aminoethanephosphonic acid [2]. In addition, serine as well as other amino acids, and ethanolamine were identified in the solution resulting from the electric discharge reaction. This motivated the extension of the condensation reaction of amino acids in aqueous solution of condensed phosphates not only to mixtures of amino acids, but also to systems containing mixtures of amino acids and other primary or secondary amines of biological interest, which are potentially derivable from prebiotic processes.

In this paper we report the reactions of amino acids and of mixtures of an amino acid (glycine or alanine) with O-phosphoethanolamine, O-phosphoserine or 2-aminoethanephosphonic acid in solutions of polyphosphates, and discuss the possible mechanism for the peptide (or phosphopeptide), and amide bond formation.

All the reactions were carried out at room temperature, in aqueous solutions containing the amino acid (0.05 or 0.1M), the phosphorus-containing amine (0.05M) and the polyphosphate (0.1 or 0.022M), at pH 11. The pH was adjusted during the course of the reaction with concentrated sodium hydroxide. The yields of dipeptide, of phospho-peptide and of amide were generally determined by comparing the peaks on the chromatogram (*Beckman* Amino Acid Analyzer) with those produced by known amounts of authentic products. The results, summarized in the table, show that a 0.1M aqueous solution of glycine and trimetaphosphate, maintained at pH 11 for 120 hours, yielded 40% of diglycine. Under the same conditions, but after 72 hours, a solution of alanine (0.1M) gave 12% of dialanine, and a solution of serine (0.1M) contained only O-phosphoserine (4%) and no detectable amount of diserine, while a solution containing a mixture of serine (0.05M) and glycine (0.05M) gave rise to 12% of diglycine, 4% of O-phosphoserine, and 2–3% of glycylserine.

When a mixture of an amino acid (0.05M glycine or alanine) and a phosphorus-containing amine, $R–NH_2$, was allowed to react under the same conditions, dipeptides and amides (crossed condensation products of the amino acid with the amine) were obtained as indicated below.

$$R–NH_2 + {}^-OOCCHR'NH_2 \xrightarrow[\text{poly-phosphate}]{\text{trimeta- or}} H_2NCHR'CONHCR'COO^- + RNHCOCHR'NH_2$$

$$R = -CH_2CH_2OPO_3H_2,\ -CH_2CH_2PO_3H_2,\ -CH(CH_2OPO_3H_2\ (COOH)$$

$R' = H$ or CH_3

When trimetaphosphate was used with glycine ($R' = H$) and phosphoethanolamine ($R = -CH_2CH_2OPO_3H_2$) or 2-aminoethanephosphonic acid ($R = CH_2CH_2PO_3H_2$), the yields of diglycine (17–22%) and of the amide (13–17%) were relatively good; with serinephosphate ($R = CH(CH_2OPO_3H_2)(COOH)$) the yield of the amide (N-glycylserinephosphate) was lower (5–6%). See Table.

The three amides (N-glycylphosphoethanolamine, N-glycyl-2-aminoethanephosphonic acid and N-glycylserinephosphate) have short elution times, all appearing at less than 30 min on the chromatogram of the amino acid analyzer. The corresponding fractions of the three amides were collected and, upon hydrolysis in 6N HCl, gave the expected amounts of glycine.

Comparable results, but in lower yields, were obtained when alanine was substituted for glycine in some of the previously mentioned experiments.

Linear polyphosphate of average chain length of 26 proved to be a less effective condensing agent. An aqueous solution of glycine (0.1M) and polyphosphate (0.022M), maintained for 72 hours at pH 11, yielded 5.7% of diglycine, while a solution of

Peptide and Amide Formation in Aqueous Solutions of Amino Acids and Amines, in the Presence of Polyphosphates, at Room Temperature, at pH ~11

(pH Maintained by Addition of Concentrated Sodium Hydroxide)

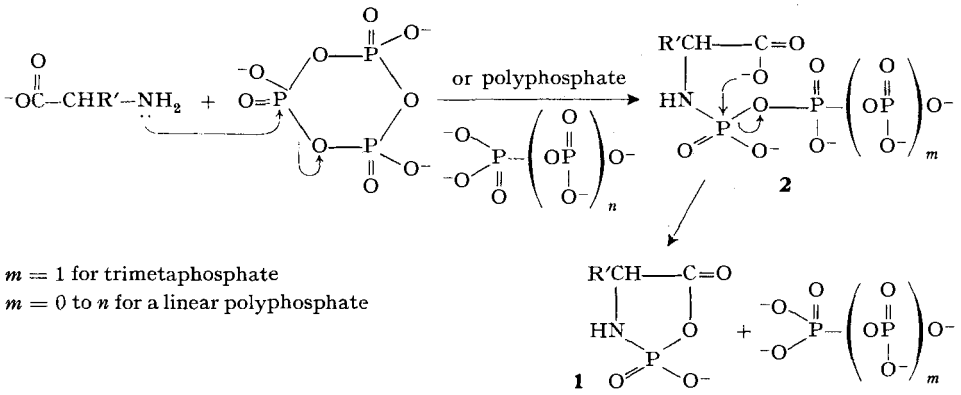
Amino Acid	Conc. M	Amine	Conc. M	Polyphosphate	Conc. M	Reaction time h	Yield Dipeptide %	Yield Amide %
Glycine	0.1			Trimetaphosphate	0.1	120	40[a]	
Alanine	0.1			Trimetaphosphate	0.1	72	12.2	
Glycine	0.05	2-Aminoethanephosphonic Acid	0.05	Trimetaphosphate	0.1	120	17	13
Glycine	0.05	O-Phosphoserine	0.05	Trimetaphosphate	0.1	120	17	5
Glycine	0.05	O-Phosphoethanolamine	0.05	Trimetaphosphate	0.1	120	22	14
Glycine	0.1			Polyphosphate $n = 26$	0.022	72	5.7	
Glycine	0.05	O-Phosphoethanolamine	0.05	Polyphosphate $n = 26$	0.022	72	3	2
Alanine	0.05	O-Phosphoethanolamine	0.05	Trimetaphosphate	0.1	72	7	4
Serine	0.1			Trimetaphosphate	0.1	72	0[b]	
Serine + Glycine	0.05 0.05			Trimetaphosphate	0.1	72	[c]	

[a] When the pH was maintained with conc. NH_4OH (instead of NaOH) the yield was 34%.
[b] 4% of O-phosphoserine is formed in this reaction.
[c] 4% of O-phosphoserine, 3% of N-glycylserine, and 12% of diglycine are formed in this reaction.

glycine (0.05M) and phosphoethanolamine (0.05M) yielded under the same conditions 3% of diglycine and 2% of N-glycylphosphoethanolamine.

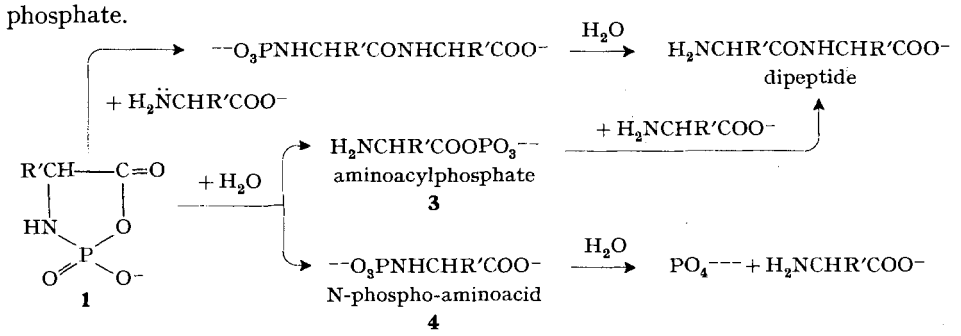
The function of the polyphosphates in promoting the condensation of amino acids may be explained in terms of reaction pathways involving the formation of cyclic and/or open acylphosphates with subsequent acylation of the amino acid group of another molecule of the amino acid by the mixed anhydride. The way in which a cyclic acylphosphoramide participates in the reaction was elucidated in collaboration with Drs. *N. Chung*, *R. Lohrmann*, and *L. E. Orgel* and shall be discussed in detail elsewhere.

The intermediate formation of a cyclic mixed anhydride **1** (4-alkyl-2-hydroxy-2,5-dioxo-1,3,2-oxaazaphospholidine) is suggested by the fact that trimetaphosphate or a long chain polyphosphate, at pH > 8–9, reacts with ammonia and amines to yield tri- or poly-phosphoramides, and under our experimental conditions would be expected to react with the amino group of the amino acid to give an N-tri-(or poly-)-phosphoramide **2**, which would easily undergo ring closure by intramolecular displacement of pyrophosphate or polyphosphate by the carboxylate group:



$m = 1$ for trimetaphosphate
$m = 0$ to n for a linear polyphosphate

This cyclic mixed anhydride **1** could react either with the amino group of another molecule of amino acid to yield a N-phospho-dipeptide which would hydrolyze to the dipeptide, or with water to form the corresponding aminoacyl phosphate **3** and N-phospho-amino acid **4**. The aminoacyl phosphates which are known to be acylating agents [3] would react further with other amino acid molecules to form the peptide bond, while N-phospho-amino acids, which do not react with amino acids (see experimental part), would be hydrolyzed to the starting amino acid and ortho-phosphate.



An alternate scheme for the formation of the cyclic mixed anhydride **1** is the nucleophilic attack of the carboxylate group of the amino acid on a P–O–P bond (of either trimetaphosphate or a linear polyphosphate), followed by an intramolecular displacement of pyro- or poly-phosphate by the amino group. Under the somewhat basic conditions of the reaction, however, attack of an unprotonated amino group on the P–O–P bond would be favored over that of the negatively charge carboxylate group.

During the early stage of the reactions, *e.g.* of glycine with trimetaphosphate, N-phosphoglycine and probably N-phosphopeptides were detected in the solution but neither cyclic nor linear acylphosphates. This is probably due to the fact that open chain acylphosphates are very labile in aqueous solutions, and five-membered cyclic acylphosphates should be even more labile (*e.g.*, cyclic dimethylenephosphate is much more labile than the corresponding open diester [4]). Since N-phosphoglycine is not transformed to a peptide under our experimental conditions, it seems reasonable to assume that the peptide bond formation proceeds through the intermediate formation of cyclic or open acylphosphates (**1** or **3**) as described in the above schemes.

Reaction pathways involving the reactive intermediate **1** or **3** would also account for the observed formation of a mixed amide, the crossed condensation product of an amino acid (glycine or alanine) and a phosphorus-containing amine $R-NH_2$, in aqueous solutions of polyphosphates. A more detailed study on the mechanism of this reaction will be reported later.

We suggest that similar peptide and amide bond formation also occurs in the electric discharge reactions of mixtures of phosphine, methane, ammonia, and water, since amino acids, phosphorus-containing amines, polyphosphates, and other P–O–P derivatives are formed *in situ* in this reaction. This view is corroborated by the fact that the chromatogram from the amino acid analyzer of an aliquot of the reaction mixture exhibited several peaks with elution times lower than 30 min. When this fraction was collected and hydrolyzed in 6 N HCl, glycine and small quantities of other amino acids were detected.

This process of condensation of amino acids, and mixtures of amino acids and amines, in aqueous solutions of polyphosphates (linear and cyclic), at room temperature and at a pH range of 7.5 to 11–12 (or higher), suggests a reasonable pathway for the prebiotic formation of peptide and amide bonds.

Experimental Section

with the collaboration of **V. H. Tashinian**

1. *Starting materials.* Glycine, alanine, serine, diglycine, dialanine, glycylserine, O-phosphoserine, and 2-aminoethanephosphonic acid were commercial products. O-phosphoethanolamine was prepared according to known procedures [5]. We thank Dr. *N. Chung, The Salk Institute for Biological Sciences,* San Diego, California, for a sample of N-phosphoglycine (Mg salt), and *Monsanto Chemical Company,* St. Louis, Missouri, for samples of sodium trimetaphosphate and polyphosphate with an average chain length of 26.

2. *Analysis of amino acids, peptides, amides and amines.* Performed on a *Beckman* 120C Amino Acid Analyzer. The elution times and colored yields of each authentic product were determined. In the case of the three amides, N-glycyl-phosphoserine, N-glycyl-phosphoethanolamine, and N-glycyl-2-aminoethanephosphonic acid, the following procedure was used: an aliquot of the reaction mixture was passed through the long column of the Amino Acid Analyzer and the fraction corresponding to the unknown peak was collected. It was then evaporated to dryness under reduced pressure, and the residue was refluxed overnight in 5 ml of 6 N HCl. The mixture was evaporated to dryness (under reduced pressure), the residue dissolved in a known amount of water, and an aliquot analyzed (Amino Acid Analyzer) for glycine and the other amines. Each of the three above fractions was also submitted to an *Edman* degradation (before hydrolysis), which showed that the N-terminal amino acid was glycine (the phenylthiohydantoin of glycine was isolated and identified by the usual procedures [6]). In this manner the three peaks with elution times of 16.5, 21.5, and 28.5 min were shown to correspond mainly to N-glycyl-phosphoserine, N-glycyl-phosphoethanolamine, and N-glycyl-2-aminoethanephosphonic acid, respectively.

3. *Condensation of amino acids and phosphorus-containing amines in aqueous solutions of cyclic or linear polyphosphates.* – 3.1. *Amino Acids.* 153 mg of sodium trimetaphosphate or 380 mg of sodium polyphosphate $n = 26$ were added to 5 ml of an 0.1M aqueous solution of the amino acid. The pH was adjusted to ~11 and maintained at that pH during the entire course of the reaction, at room temperature. Aliquots of the reaction solution were analyzed (Amino Acid Analyzer) at given times and the yields of dipeptide or of phosphorylated material were determined by comparing the area of the peaks on the chromatogram to those obtained with known amounts of authentic products. The results are summarized in the table.

3.2. *Amino acids and phosphorus-containing amines.* 5 ml of a solution containing the amino acid (0.05M), the phosphorus-containing amine (0.05M) and 153 mg of trimetaphosphate (0.1M) or 380 mg of polyphosphate $n = 26$, were adjusted to pH ~11 with conc. NaOH and maintained at that pH, at room temperature, for 72 hours. The yields of dipeptide were determined as above (3.1), and those of amide as indicated under 2.

4. *Behaviour of N-phosphoglycine in aqueous solution and in an aqueous solution of glycine.* – 4.1. *In H_2O.* A 0.1M solution of N-phosphoglycine was maintained, at room temperature, at pH 11 for 120 hours. The analysis of an aliquot indicated a yield of 50% of glycine (and orthophosphate) but no diglycine. Elution time of N-phosphoglycine: 13 min.

4.2. *An aqueous solution of N-phosphoglycine (0.05M) and glycine (0.05M)* maintained, at room temperature, at pH ~11 for 120 hours, gave no dipeptide and about 40% of the N-phosphoglycine was hydrolyzed.

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159. Über das makrocyclische Spermidinalkaloid Inandenin[1])

138. Mitteilung über Alkaloide [1]

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(24. VI. 70)

Summary. The novel spermidine alkaloid inandenine was isolated from *Oncinotis inandensis Wood et Evans.* It was found to be a mixture of two isomers A and B, which could not be separated hitherto. Chemical and mass-spectrometric investigations revealed that both alkaloids possess the macrocyclic 21-ring structure **3a** and **3b**. These two formulae differ only with respect to the position of the ketogroup. Structural elements of the two bases are 9-oxo- and 10-oxo-palmitic acid, respectively, and spermidine.

[1]) Teil der geplanten Dissertation von *H. J. Veith.*