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To be published in Proceedings of the
National Academy of Science

UCRL-11473

UNIVERSITY OF CALIFORNIA

Lawrence Radiation Laboratory
Berkeley, California

AEC Contract No. W-7405-eng-48

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May 1964

c. Nat. Acad. Sci.
/13/64

CYANAMIDE: A POSSIBLE KEY COMPOUND IN CHEMICAL EVOLUTION*

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The results reported from many laboratories over the past decade[1-4] make it clear that all of the necessary biological monomers could have been formed on the primitive earth. By "biological monomers" we mean compounds such as the amino acids (on the way to proteins), the simple sugars (on the way to carbohydrates and nucleic acids), and the purines (on the way to nucleic acids). There is no doubt that such compounds would have been formed on the earth under its assumed early atmosphere (composed mostly of CH_4, NH_3, N_2, H_2O and H_2).

There now looms the problem of finding reasonable mechanisms by which the biological monomers may have condensed to form the polymers under primitive earth conditions. The condensations under consideration (amino acids to proteins,, sugars to carbohydrates, sugars to sugar phosphates, purines to nucleosides, and nucleosides to nucleotides) are all dehydration reactions, and a number of suggestions have been made as to how they took place. One of them is that a dehydrating conditions could have arisen in evaporating ponds.[5] A second is that the reaction may have taken place upon contact with suitable mineral surfaces.[6] However, it seems to us that a more likely approach to the problem of biological polymer appearances would be to find conditions that would result in dehydrations in dilute aqueous media.

In considering compounds that can effect dehydration polymerizations our attention was drawn to HCN plus UV light, and to the carbodiimides.[7] The substituted carbodiimides are widely used to effect dehydration condensations, for example, of amino acids to peptides,[8,9] and of alcohols (including sugars) with phosphoric acid to form phosphate esters;[10] in both these examples, water may be present in the reaction mixture. However, there is little reason to suppose that any significant quantity of the dialkyl carbodiimides were present on the primitive earth. A more likely candidate is cyanamide itself, a compound which hydrolyzes relatively slowly, that was probably present on the primitive earth, and that is a tautomer of carbodiimide ($H_2N-C\equiv N \rightleftharpoons HN=C=NH$).

We have tested the effect of cyanamide, or its dimer, in promoting the following condensations in dilute aqueous solutions: (a) glucose plus orthophosphoric acid to glucose-6-phosphate; (b) adenosine plus orthophosphoric acid to adenosine-5'-phosphate; (c) orthophosphoric acid to pyrophosphoric acid; (d) glucose to disaccharides; and (e) amino acids to dipeptides.

Materials and Methods. - In all our experiments (with one exception stated below) the cyanamide was used in the form of its dimer, $H_2N-C(=NH)NHCN$. This compound, labeled "cyanamide", was obtained from Eastman. Its melting point (209°) and its molecular weight (86, by Rast determination) corresponded to the dimer. The monomer, used in some of our experiments, was freshly prepared for us by Dr. Horst Koeller by the dehydrosulfurization of thiourea by HgO. Its melting point was 43°.

For the glucose-6-phosphate experiments, 0.64 μmoles of chromatographically purified glucose (containing 1.6 μc of C^{14}) and 1.0 mg of cyanamide dimer was dissolved in 1 ml of 0.01 M H_3PO_4. The solution (pH 2) was allowed to stand for 19 hours at room temperature. Carrier glucose-6-phosphate was then added and the entire solution was paper chromatographed (first solvent system: methanol-16 N NH_4OH-water (6:1:3 by volume); second solvent: methanol-ethanol-water (9:9:2 by volume)). The sugars and sugar phosphates were located on the completed chromatogram with a benzidine spray; radioactivity was located by exposure of X-ray films to the chromatogram. An identical experiment was performed using 0.5 mg of freshly prepared monomer in place of the cyanamide dimer.

The possible formation of adenosine-5'-phosphate was determined as follows: Chromatographically-purified C^{14}-labeled adenosine (0.29 μmoles, 0.38 μc), 2.6 ml of 0.01 M cyanamide (dimer), and 0.4 ml of 0.06 M H_3PO_4, (final pH 2.2) were allowed to stand for 4.5 hours at room temperature. Carrier adenosine-5'-phosphate (AMP) was added and the mixture paper chromatographed (first solvent: organic phase from a 4:1:5 n-butanol-acetic acid-water mixture; second solvent: isopropanol-water (4:1)). The position of the AMP was located by a shadowgram, the radioactivity by X-ray film. Another experiment was performed with all conditions identical with those above except that the solution was adjusted to pH 7 with NaOH.

The production of inorganic pyrophosphate from orthophosphate was examined by allowing 1 ml of a solution of 10^{-2} M H_3PO_4 (pH 2.1) ($H_4P_2O_7$-free) and 10^{-2} M cyanamide (dimer) to stand for 10 hours at room temperature. The solution was then paper chromatographed (one-dimension) with the n-butanol-acetic acid-water solvent. The phosphates were located by the ammonium molybdate spray.

Results and Discussion. - The results recorded in Table I demonstrate that cyanamide can induce the formation of the phosphomonoester between glucose and phosphoric acid. The dimer appears to be more effective than the monomer in accomplishing this reaction. While cyanamide dimer can induce the formation of pyrophosphate from orthophosphate, the data of Table I also demonstrate that pyrophosphate in the absence of cyanamide dimer does not phosphorylate glucose in dilute aqueous medium at room temperature. Our chromatograms also showed the presence of two other labeled products (possibly sugar phosphate), but their identity has not been established. It is interesting to note that the phosphorylation reaction has occurred very prominently on the primary alcohol group of the glucose. The relative speed of phosphorylation of the others remains to be determined.

The corresponding dehydration reaction has been demonstrated with the formation of adenosine-5'-phosphate from adenosine and orthophosphoric acid under the influence of the cyanamide dimer. As far as we are aware, this is the first report of the formation of AMP under the sole influence of compounds and conditions such as would be expected to exist on the primitive earth. While this reaction occurs at pH 2, it seems to be very slow, if it occurs at all, at neutral pH. The significance of this pH effect with regard to the mechanism of the reaction will be discussed below.

We have shown that, under presumed primitive earth conditions, cyanamide dimer will condense inorganic orthophosphate to give the pyrophosphate linkage. This resembles the reaction demonstrated by Todd in the formation of an adenosine-uridine pyrophosphate and of cytidine-5'-pyrophosphate.[11] The

TABLE I

Formation of Glucose-6-Phosphate

Compounds Present in Reaction Mixture

Glucose	Cyanamide Monomer	Cyanamide Dimer	H_3PO_4	$H_4P_2O_7$	Formation of Glucose-6-Phosphate
+	+	-	+	-	+ (1.5% yield from glucose)
+	-	+	+	-	+ (2.4% " " "
+	-	-	+	+	none detectable

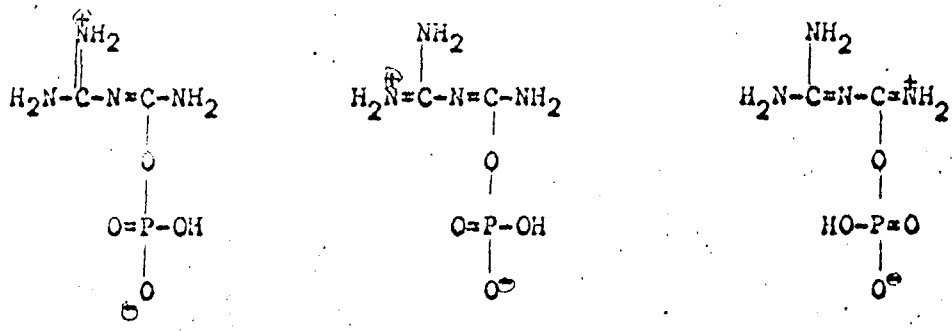
conditions used for this reaction, however, were considerably different from our own. The reaction was carried out in a very concentrated solution and at elevated temperatures. Ours was carried out in dilute solution at room temperature.

Our attempts to demonstrate the dehydration polymerization of glucose by the cyanamide dimer have indicated that ultraviolet light is required to induce some reaction of this type. The precise character of the product is not yet known to us. We have also subjected dilute solutions of amino acids to cyanamide dimer and ultraviolet light. Our early results suggest that these conditions lead to the formation of peptides. It has previously been demonstrated that in nonaqueous media and at elevated temperatures (80-100°) dipeptides are formed by the action of cyanamide on acylated amino acids.[12]

Cyanamide dimer was unable to accomplish the dehydration of adenine with ribose to produce adenosine, either by itself or with the assistance of ultraviolet light. This is to be contrasted with the successful formation of adenosine in the presence of ultraviolet light and orthophosphate,[3] an experiment which we have confirmed.

While no details of the mechanism of these dehydration reactions are yet available to us, there are two experimental facts which might give some indications. These are that the dimer seems to be more effective in the dehydration reaction than the monomer, and, second, that the reaction goes best at low pH. These two, taken together, suggest that it is the carbodiimide form which is the effective agent and that it is effective by virtue of its ability to add a phosphoric acid molecule across the carbo-

diimide double bond. It is known that in the crystals of the dimer there is a large component of the tautomer containing the substituted carbodiimide group, $H_2N-C(=NH)-N=C=NH$.[13] Thus, we expect with the dimer more of an amidinium phosphate intermediate which, presumably, is involved in the dehydration reaction. Three of the principal resonance forms would be:



Footnotes and References

* The work described in this paper was sponsored by the U.S. Atomic Energy Commission.

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