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Gary Steinman, Dean H. Kenyon and Melvin Calvin

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Dehydration Condensation in Aqueous
Solution

Earlier studies have demonstrated that dicyandiamide (DCDA), the dimer of cyanamide, can successfully promote the dehydration condensation of (i) glucose and orthophosphate to give glucose-6-phosphate,¹ adenosine and orthophosphate to give adenosine-5'-monophosphate,¹ (iii) orthophosphate to give pyrophosphate,¹ and (iv) alanine to give alanylalanine and alanylalanylalanine.² These reactions were carried out in dilute aqueous solution in the dark.* These experiments were designed to demonstrate one possible means by which such compounds could have been formed on the prebiotic earth, thus providing materials needed for the origin of living systems. Dicyandiamide itself could have been present on the primitive earth as was demonstrated with the ultraviolet irradiation of cyanide solution.³

Guanylurea was demonstrated as a concomitant product of dipeptide synthesis using dicyandiamide in the following experiment: A stock aqueous solution was prepared containing HCl (0.02 N), DCDA (0.02 M), and alanine (0.02 M). A 50 λ aliquot of this solution was added to 50 λ of an aqueous solution containing 0.5 μ c of ¹⁴C-labelled alanine. Also, 50 λ of the stock solution was added to 50 λ of an aqueous solution which contained 0.6 μ c of ¹⁴C-labelled dicyandiamide. The solutions remained in the dark at room temperature for 20 hrs. Each was directly applied to a

* It was also demonstrated that the combination of ultraviolet light and dicyandiamide could promote the synthesis of dipeptides.¹ This observation has since been confirmed by other investigators.⁴



separate sheet of Whatman No. 1 paper. Standard samples of guanylurea and alanylalanine were placed in parallel to the test samples on the appropriate sheets. The chromatogram containing labelled alanine was run with water-saturated phenol: conc. NH_4OH (200:1) as solvent whereas the other, containing labelled DCDA, was run with n-butanol:ethanol:water (4:1:1) as solvent. The standard sample of dipeptide was located with ninhydrin, the guanylurea was found with a nitroprusside spray, and the labelled products were located by means of X-ray film. The results indicated that 0.51% of the original alanine was converted to the dimer. After subtracting the amount of guanylurea formed by direct hydrolysis of DCDA, it was found that the amount of excess guanylurea formed was equal to the number of peptide bonds formed.

In an attempt to find compounds which might act as more effective dehydrating agents than dicyandiamide ($\text{NH}_2\text{-C(=NH)-NH-CN}$), the possible use of dicyanamide (NC-NH-CN) was investigated. Sodium dicyanamide (NaDCA) is readily synthesized from disodium cyanamide and cyanogen bromide.⁵ The experiment described above using labelled alanine was repeated using sodium dicyanamide in place of dicyandiamide. A yield of 1.58% of the ^{alanine}dimer was observed.

To investigate the application of dicyanamide (DCA) in the synthesis of polypeptides, as well as the relative reactivity of amino acids and peptides, the following experiment was performed: A 100 λ aqueous solution was prepared containing 10 μc of glycine-2-¹⁴C (0.12 M), triglycine (0.12 M), NaDCA (0.12 M) and HCl (0.12 N). A similar solution was prepared containing no NaDCA. After remaining 21 hrs in the dark at room temperature,



20 λ aliquots were removed from each solution and were spotted on strips of Whatman No. 1 filter paper together with standards of diglycine and tetraglycine. These samples were then subjected to low voltage horizontal electrophoresis following the method of Carnegie and Syngé.⁶ X-ray film located the labelled products and ninhydrin identified the carriers. Radioactive bands coinciding with the standard diglycine and tetraglycine were observed. The determination of the activity associated with each band indicated that the yield of tetraglycine was about 6.1% and that of diglycine was about 2.3%, both based on initial glycine. About 13.3% of the labelled glycine appeared in as yet unidentified products.

The use of dicyanamide in phosphorylations was also examined. A 95 λ aqueous solution was prepared containing 2 μ c of ¹⁴C-labelled glucose (0.08 M), H₃PO₄ (0.08 M) and NaDCA (0.1 M). A similar solution was prepared containing DCDA in place of NaDCA. After 40 hrs at room temperature in the dark, each solution was applied to Whatman No. 1 paper and chromatographed in parallel with a standard glucose-6-phosphate sample using 95% ethanol:water:formic acid (70:29:1) as solvent. The standard was located with benzidine spray. The results indicated that DCA is about three times as effective in the production of glucose-6-phosphate as is DCDA, with the yield using DCA being 1.9%.

The possible catalytic role of mineral surfaces during chemical evolution has been suggested.⁷ Akabori has used kaolinite in the production of glycine peptides from aminoacetonitrile.⁸ Miller has been able to synthesize pyrophosphate using apatite and potassium cyanate.⁹ Since it is



known that the hydroxyl groups on the surface of kaolin can be replaced by H_2PO_4^- ions,¹⁰ the possible enhancement of DCDA-mediated phosphorylations by kaolin was investigated.

The production of pyrophosphate from orthophosphate using DCDA, as reported earlier,¹ was repeated. A 100 λ solution was prepared containing 3.6 μc of ^{32}P -labelled phosphoric acid (0.1 M) and DCDA (0.1 M). A similar solution was prepared without the DCDA. After 40 hrs, both samples were chromatographed on Whatman No. 1 paper using isopropanol:water:TCA: NH_4OH (75:25:5:0.25) as solvent. The products were located with X-ray film and eluted. Scintillation counting indicated a production of 0.2% pyrophosphate. This figure has been corroborated by another laboratory.¹¹ Next a 1 ml solution was prepared containing 10.8 μc of ^{32}P -labelled phosphoric acid (0.1 M) and DCDA (0.1 M). Another solution was prepared in the same manner but contained no DCDA. Each solution was added to 8 mg of kaolin (the X-ray powder pattern of this kaolin compared well with the periodicities reported by Brindley and Robinson¹²) and both were continually stirred for 40 hrs. The kaolin was then removed by centrifugation, and a 100 λ aliquot of each solution was analyzed for pyrophosphate as described above. Kaolin alone did not appear to promote pyrophosphate synthesis, but the combination of kaolin and DCDA gave a yield of 1.8%. This increase in pyrophosphate was also observed with the ^{colorimetric} method of Karl-Kroupa.¹³

The use of the combination of kaolin and DCDA in the production of adenosine triphosphate (ATP) was next examined. A 1 ml solution containing ADP (0.01 M), H_3PO_4 (0.01 M) and DCDA (0.01 M) was prepared. A similar

solution containing no DCDA was also prepared. Each solution was stirred in the presence of 8 mg of kaolin for 24 hrs. The kaolin was then removed by centrifugation and the solution was finally neutralized with NaOH.

Both samples were analyzed by the luciferase method of Strehler and Totter¹⁴ where the total solution was added to 250 λ of the buffered enzyme solution and/ observed in an Aminco spectrophotofluorometer at 556 m μ . The yield of ATP with DCDA and kaolin was of the order of 0.5% while in the absence of kaolin it was less than 0.03%.

Kaolin and the clay mineral, montmorillonite, do not appear to enhance the yield of dipeptide formed with the aid of DCDA.

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GARY STEINMAN

DEAN H. KENYON

MELVIN CALVIN

Lawrence Radiation Laboratory,
Department of Chemistry, and
Laboratory of Chemical Biodynamics
University of California
Berkeley, California

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