

FORMATION OF CYANATE AND CARBAMYL PHOSPHATE BY ELECTRIC DISCHARGES OF MODEL PRIMITIVE GAS

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(Received 28 July, 1981; in revised form 28 October, 1981)

Abstract. A mixed gas of nitrogen, carbon dioxide, and hydrogen was discharged over 100 ml of 0.2 M NaHCO₃ solution in a 5 liter discharge apparatus which simulates the primitive Earth. The formation of cyanate, which is one of the possible primitive condensing agents, was demonstrated by the detection of [Cu(Py)₂](NCO)₂ that was formed by the addition of copper sulfate-pyridine reagent to the solution. In a series of experiments the partial pressures of nitrogen and carbon dioxide in the starting gas were fixed at 10 cm Hg and 20 cm Hg, respectively, whereas that of hydrogen was varied between 5, 10, 15, 20, 25, and 30 cm Hg. The discharges were continued for one week. The rate of appearance of cyanate was strongly dependent upon the partial pressure of hydrogen. The maximum rate of the production of cyanate at the initial stage of the discharge was in the case of 10 cm Hg of hydrogen, in which condition the starting gas is in a predominantly oxidized state. In this case the concentration of cyanate reached about 0.012 M after one day. Another discharge experiment was carried out with 0.2 M phosphate solution, and the production of carbamyl phosphate was demonstrated through the formation of ATP by the incubation of the discharged solution with ADP and carbamyl phosphokinase.

A model of chemical evolution in the early stage involves two processes, the formation of simple biomolecules such as amino acids, nucleic-acid bases, and riboses which would have been accumulated in primitive ocean, and the polymerization of these monomers through the dehydration reaction effected by active molecules produced from primitive gases (Miller and Orgel, 1974). The latter reaction has been investigated using various condensing agents which were expected to have been produced on the primitive Earth. However, as far as we know, these condensing agents have not been confirmed to be formed from model primitive gases, with the exception of dicyandiamide (Schimpl *et al.*, 1965). Cyanate was first noticed by Jones and Lipmann (1960) as the active substance which forms carbamyl phosphate, an energy-rich phosphate carrier, by spontaneous condensation with phosphate. Later, phosphorylation experiments using cyanate were performed by Miller and Parris (1964), and Lohrmann and Orgel (1968).

Recently we reported the production of AMP and pyrophosphate in aqueous solution of adenosine and phosphate by electric discharges of model primitive gas over the solution in a discharge apparatus simulating prebiotic chemical evolution (Yamagata *et al.*, 1979; Yamagata *et al.*, 1981). In the experiment, it was expected that if the condensing agent is cyanate, the dehydration condensation could be continued through a long period without supply of gases because cyanate is hydrolyzed to gaseous components, ammonia and carbon dioxide. We report here the experimental study of the formation of cyanate and carbamyl phosphate by electric discharge of model primitive gas.

The experiment was performed using the apparatus which was constructed for the study of dehydration condensation described above. In each of the experiments the 5-liter discharge vessel was prepared with 100 ml of 0.2 M NaHCO₃ solution (pH 8.1) and the gas mixture of nitrogen, carbon dioxide, and hydrogen given in Table I. The solution was kept at about 30°C through the experiments. Seven parallel discharges were induced in each gap between a center co-electrode and seven other electrodes around it.

TABLE I
Starting gas compositions

Starting gas	Run no.							
	1	2	3	4	5	6	7	8
N ₂ (cm Hg)	10	10	10	10	10	10	7	14
CO ₂ (cm Hg)	20	20	20	20	20	20	14	28
H ₂ (cm Hg)	5	10	15	20	25	30	14	28

Two ml of the solution was pumped out each time and mixed with 1 ml of the reagent which was prepared with copper sulfate and pyridine (Martin and McClelland, 1951). After the solution stood at 18°C for 10 min, the complex of copper formed with pyridine and cyanate, [Cu(Py)₂](NCO)₂, was extracted four times with 1 ml of chloroform, and the concentration of cyanate was estimated by the absorption at 690 nm (λ_{\max} is 690 nm rather than the 680 nm described in the original paper (Martin and McClelland, 1951)). The linearity between the OD₆₉₀ and the concentration of cyanate solution was confirmed with NaNCO. The experimental results for the formation of cyanate in runs 1–5 are given in Figure 1 (the result in run 6 was similar with that in run 5). In runs 1–6 the pressure of nitrogen and carbon dioxide in the starting gas mixture was fixed as shown in Table I, so that nitrogen and carbon sources are constant in the series, and only the pressure of hydrogen was varied. In runs 1 and 2, in which the starting gas in the predominantly oxidized condition, the yield of cyanate reached a maximum at one day of discharge, and then decreased rapidly. This may be explained by a change of the gas composition towards the more oxidized condition because of the trapping of ammonia into the solution as described before (Yamagata *et al.*, 1979; Yamagata *et al.*, 1981). In fact, after the fourth day of discharge in run 1, the chloroform extract showed a different absorption spectrum from that of [Cu(Py)₂](NCO)₂.

The rate of increase of the cyanate concentration at the initial stage in Figure 1 should reflect the efficiency of the cyanate formation from the gas of the starting composition, because the gas composition and the solution would change less from the starting conditions for the initial short period. The figure shows that the maximum efficiency is in the case of 10 cm Hg of hydrogen, in which the gas composition is in a largely oxidized state. Another series of experiment (runs 7 and 8)

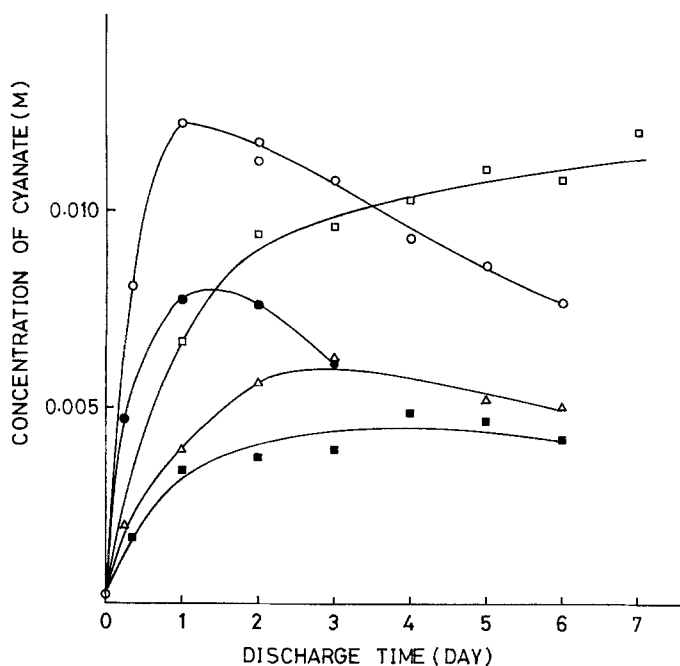


Fig. 1. Dependence of concentration of cyanate on the discharge time in runs 1-5. ● run 1, ○ run 2, □ run 3, △ run 4, ■ run 5.

was carried out with the gases of the same proportion of their partial pressure but of different total pressures. The results were almost the same for these three runs and it indicates that the formation of cyanate is independent of the total pressure within the present experiments.

Further, an experiment was performed for the formation of carbamyl phosphate which is spontaneously produced from cyanate and phosphate by an equilibrium reaction (Jones and Lipmann, 1960). In our experiment 100 ml of 0.2 M phosphate solution (0.4 g KH_2PO_4 + 3.0 g K_2HPO_4 , pH 7.6) and gas mixture of N_2 (10 cm Hg), CO_2 (20 cm Hg), and H_2 (15 cm Hg) was prepared in the discharge vessel. The discharge was carried out in the same manner as described above. Two ml of the discharged solution was mixed with 5 mg of ADP and about 5 unit of carbamyl phosphokinase (Sigma Chem. Co., one unit forms 1.0 μM of ATP from ADP and carbamyl phosphate per minute at pH 8.3 and 37°C). ADP was purified on a DEAE-Sephadex column to eliminate ATP and other nucleotides. After the incubation at 37°C for several hrs (1 h ~ 6 h) the reactant was applied to a Sephadex G-10 column (2.64 × 65 cm) and was eluted with 0.1 M sodium citrate buffer of pH 4.4. On the chromatogram a peak appeared corresponding to ATP, which indicates the production of ATP by carbamyl phosphokinase from ADP and carbamyl phosphate formed in the discharge vessel. ATP was also confirmed by silica gel TLC. The control experiment with the starting solution showed no peak of ATP.

The considerable dependence of the concentration of cyanate on the gas composition may explain the strong dependence of the AMP production on the composition of the starting gas in the previous experiment (Yamagata *et al.*, 1979), and this suggests the possibility of cyanate as the condensing agent involved.

Acknowledgement

We thank Dr Inomata for his suggestion on the analysis of cyanate and his reading of the manuscript.

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