PREBIOTIC NUCLEOTIDE SYNTHESIS-DEMONSTRATION OF A GEOLOGICALLY PLAUSIBLE PATHWAY

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Abstract. Mineral phosphate (apatite) is activated for the synthesis of nucleotides when dilute solutions containing nucleoside and ammonium oxalate are evaporated in its presence. A natural, igneous fluorapatite was found to be even more effective in nucleotide synthesis than the more soluble hydroxylapatite. The phosphorylation is considerably more efficient if urea or cyanamide is also present. Hydrolysis of solutions of cyanogen to form oxalate and urea among other products is a spontaneous process that provides a geologically plausible model for nucleotide synthesis on the primitive earth.

1. The Apatite-Oxalate Model

The theoretical background for this model is to be found in Schwartz (1971) and Schwartz and Deuss (1971). It was predicted that the problem of the insolubility of apatite (Gulick, 1955; Miller and Urey, 1959) might be overcome by means of the complexing of calcium by oxalate, particularly at slightly reduced pH. The applicability of the pathway to the phosphorylation of uridine was demonstrated in model 'evaporating pond' experiments (Schwartz, 1972). As part of a more systematic study of the phosphorylation process, we have examined the phosphorylation of thymidine-2-^{14}C(5mCi mmole^{-1}). Solutions which were 10^{-4} M in nucleoside and 10^{-3} M in ammonium oxalate were evaporated and heated in the presence of 10 mg of hydroxylapatite or fluorapatite in 15 ml centrifuge tubes at 90 °C for one week. In those experiments in which an organic reagent was also added, its concentration was 10^{-3} M.

Quantitative yields were determined by electrophoresis of water extracts of the residues (0.02 M sodium acetate containing 0.008 M EDTA, pH 6.3, 18 V cm^{-1} for 2 hr). Liquid scintilation counting was carried out on 1.0 cm sections of each strip. Table I summarizes some of the results. Peaks representing components more highly anionic than TMP have been summed and are referred to as 'higher products'. The nature of these products is described in the caption to Figure 1, which also illustrates another method of separation.

In contrast to the results obtained for the slow evaporation of large volumes of dilute uridine solutions (Schwartz, 1972), Table I shows no positive effect of the addition of imidazole. This observation is in agreement with the results of Stillwell *et al.* (1972) in a study of the phosphorylation of glucose. It should be noted that the rate of evaporation of 1 ml volumes is such that the tubes appear to be dry by 16 hr. The previous experiments on the phosphorylation of uridine were carried out in volumes of 2.5 l, requiring from 4 to 7 days for evaporation. Besides possible differences in results due to evaporation rates, we have also noted differences due to the nature of the nucleoside and this effect is now under further investigation.

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TABLE I

Phosphorylation of thymidine with apatite[a]

Addition	Apatite[b]	TMP (%)[c]	Higher products (%)	Total yield (%)
Dicyandiamide	FA	30.3 (±2.3)	50.6 (±3.4)	81
Dicyandiamide	HA	45.7 (±0.9)	19.8 (±5.8)	66
Urea	FA	24.4 (±1.8)	10.8 (±1.8)	35
Urea	HA	18.5 (±0.4)	2.2 (±0.3)	21
Imidazole	FA	18.2 (±0.5)	2.0 (±0.1)	20
Imidazole	HA	9.5 (±2.1)	–	10
None	FA	16.8 (±2.4)	5.4 (±0.8)	22
None	HA	15.5 (±2.3)	1.5 (±0.2)	17
Cyanogen hydrolysates:				
Solution A	HA	28.0 (±1.2)	54.5 (±2.2)	83
Solution B	HA	27.4 (±0.8)	51.9 (±0.9)	79

[a] For conditions see text. All yields are based on conversion of thymidine and are averages of four replicate experiments.

[b] HA is synthetic hydroxylapatite (Riedel-de Haën). FA is an igneous, macrocrystalline fluorapatite (Durango), ground in a Tema mill to approximately the same particle size range as the hydroxylapatite.

[c] Yields of TMP represent mixtures of 3′ and 5′ nucleotides.

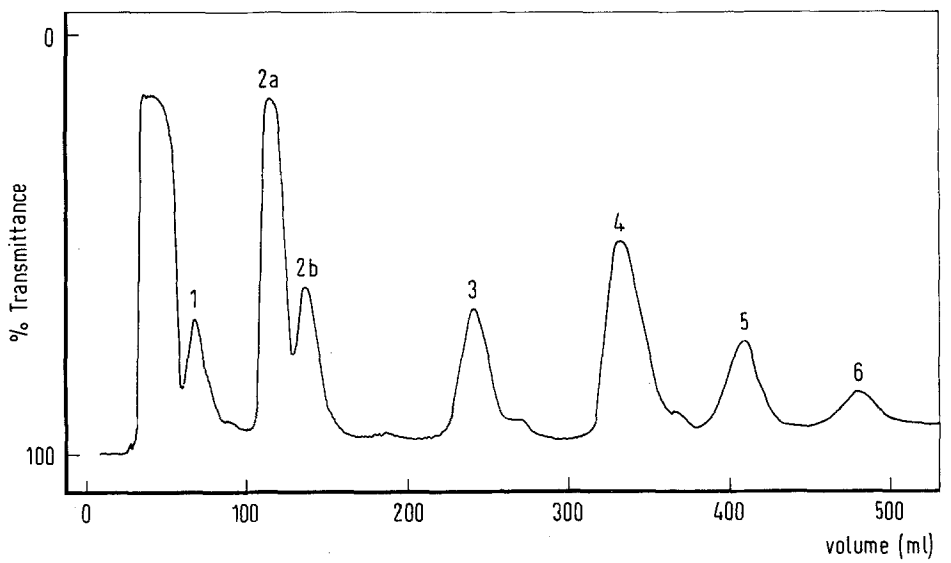

Fig. 1. Initial fractionation of the products of the phosphorylation of thymidine with hydroxylapatite in the presence of dicyandiamide. DEAE-Sephadex (1.27 × 18 cm); linear gradient of 0.05 to 0.6 M triethylammonium bicarbonate at pH 7.6 (800 ml). Peaks 2a and 2b (TMP) are thymidine 5′- and 3′-monophosphate, respectively (molar ratio approximately 5:1). All higher peaks appear to be mixtures of products. One of the components in peak 3 appears to be thymidine-5′-pyrophosphate (ppT). The predominant product in peak 4 is thymidine-3′, 5′-dimonophosphate (pTp). Work is still underway on the full characterization of these mixtures.

Of particular interest in Table I is the effect of utilizing a natural, igneous fluorapatite in place of synthetic hydroxylapatite. Not only were yields generally increased, but a higher proportion of multiply phosphorylated products was obtained. Since the particle size distributions of the two preparations were similar, it is possible that differences in surface structure may be responsible. It is especially noteworthy that increasing the proportion of hydroxylapatite causes a decrease in yield, particularly of multiply phosphorylated products (Figure 2). This result is suggestive of the transphosphorylation of intermediate polyphosphates (Krane and Glimcher, 1962). The most significant observation, however, is the failure of the natural mineral to show the same inhibitory behavior (Figure 2). Whether this property is a general one for fluorapatites or a peculiarity of this particular mineral is yet to be established.

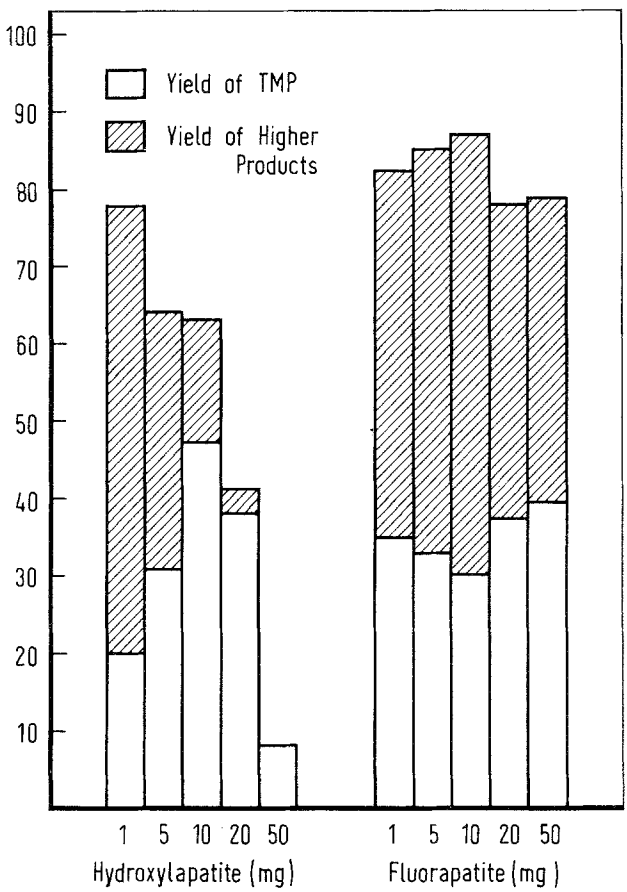

Fig. 2. The effect of quantity and source of apatite on the products of a phosphorylation reaction. Phosphorylation was carried out with the indicated quantity of apatite added to 1 ml of 10^{-4} M thymidine-2-^{14}C (10^{-4} M) containing 10^{-3} M dicyandiamide and 10^{-3} M ammonium oxalate. Reaction conditions and quantitative analyses were as described in the text. Each bar of the graph represents the average of four replicate experiments.

2. Phosphorylation by Cyanogen Hydrolysates

Hydrogen cyanide and cyanogen have been repeatedly implicated as key intermediates in prebiotic organic synthesis (see, for example: Miller, 1957; Sanchez *et al.*, 1967; Lohrman and Orgel, 1968). The presence of these substances in interstellar dust clouds and in comets (Oró, 1972) increases the probability of their having played a central role in the formation of primitive organic matter. Ammonium oxalate is a product of the hydrolysis of cyanogen (Sidgwick, 1942). Recently, oxalate has been reported to be formed, together with large amounts of urea, in the oligomerization of hydrogen cyanide and also (after hydrolysis) from cyanogen solutions (Ferris, *et al.*, 1973a, b).

We have tested the possibility of the phosphorylation of nucleosides with apatite using only cyanogen solutions as starting materials (that is, without addition of oxalate or condensing agent). Some results are included in Table I. Solution A, which was initially 0.060 M in cyanogen, was shaken at 55 °C for 1 week. The solution was filtered and 1 ml samples of the filtrate were evaporated in the presence of hydroxylapatite and thymidine-2-^{14}C in the manner already described. A portion of the filtrate was also evaporated to dryness under vacuum to remove any remaining free cyanogen. This residue was reconstituted to the original volume and titrated with ammonia to the original pH (from 4.7 to 6.6). The reconstituted solution (B) was also utilized for phosphorylation experiments. The results indicate that free cyanogen is not acting as a condensing agent in this system, although both solutions A and B serve as efficient condensing agents.

Trimethylsilyl derivatives of dried samples of the cyanogen filtrates were prepared for gas chromatographic analysis (Butts, 1972). Figure 3 is a photograph of a typical chromatogram. The identities of the labeled peaks were tentatively established by coinjection of standard samples. In agreement with the result of Ferris *et al.* (1973) urea was the major product identified. The concentrations determined were: urea, 8.4 mM; oxalate, 1.1 mM; oxamate, 0.8 mM and oxamide, 0.5 mM.

Note that the concentration of oxalate is of the same order of magnitude as that usually employed in our other phosphorylation experiments. The large amount of urea present and the expected formation of ammonium phosphate and subsequent pH reduction as the result of the evaporation of ammonium oxalate-apatite mixtures, makes it likely that the final phosphorylation is similar to those reported for ammonium phosphate-urea mixtures (Beck *et al.*, 1967; Lohrmann and Orgel, 1971; Bishop *et al.*, 1972). Of course, the possible contribution of other products, not yet identified, cannot be ruled out.

3. Prebiotic Abundance of Oxalate

In view of the facile formation of oxalate from cyanogen or hydrogen cyanide, it may be superfluous to mention other chemical evolution experiments which have also led to its synthesis. However, in this connection the experiment of Hasselstrom and

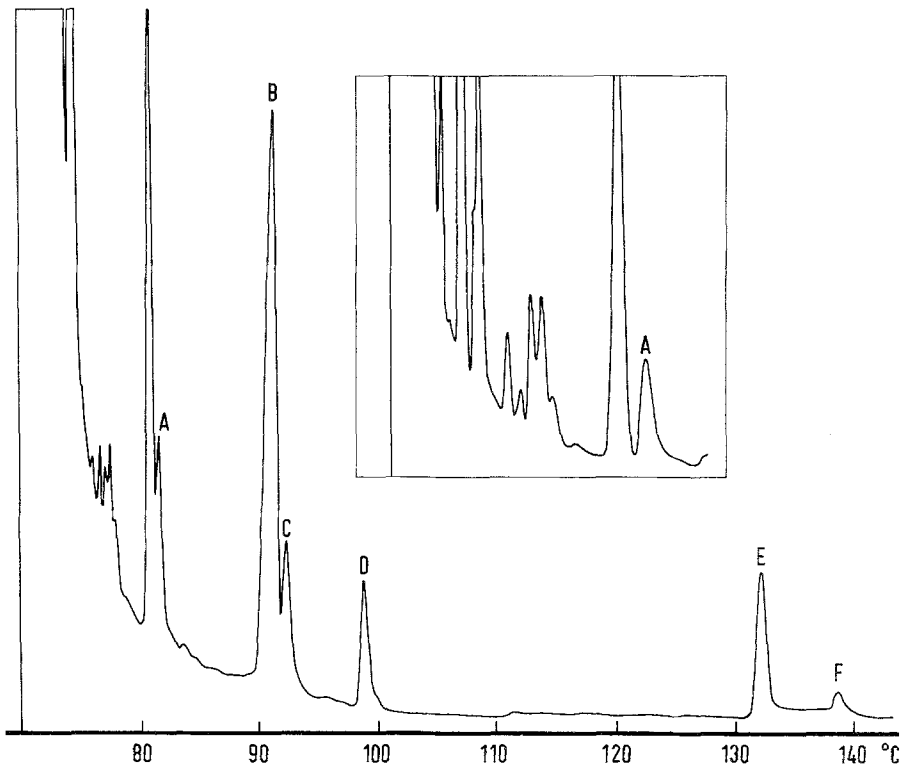

Fig. 3. Gas chromatogram of trimethylsilyl derivatives of products of the hydrolysis of cyanogen. The column (1.5 m × 3 mm I.D.) was packed with 3% OV-1 on Chromosorb G; nitrogen carrier gas at 26 ml min^{-1}; linear temperature program from 70° to 250 °C at 2° min^{-1}. Peak A is oxalic acid, B is urea, C is oxamic acid and D is oxamide. Peaks E and F have not yet been identified. The insert shows a better separation of oxalic acid from a reagent peak, performed isothermally at 70 °C.

Henry (1956) is of interest since it demonstrated the production of oxalate even under non-reducing conditions, by ultraviolet irradiation of carbonate solutions. However, the production of oxalate from the cosmically abundant CN moiety certainly seems more pertinent. In this context, it may not be amiss to point to the little-quoted fact that oxalic acid may be the singly most abundant organic compound to be identified in water extracts and hydrolysates of lunar samples (Gehrke *et al.*, 1972).

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