

AMP SYNTHESIS IN AQUEOUS SOLUTION OF ADENOSINE AND PHOSPHORUS PENTOXIDE

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Abstract. Possible formation of a P_4O_{10} molecule in magma, the stability of the molecule in hydrous volcanic gas at high temperatures and a possible prebiotic phosphate cycle were discussed in relation to chemical evolution. To demonstrate the utility of phosphorus pentoxide as a phosphorylating agent, aqueous solutions of adenosine (0.02 M) and phosphorus pentoxide (0.2 M) were incubated at 37°C for 5 months. The pH of the solutions was adjusted every day or every few days to each fixed value (9.0, 10.5, 11.5, 12.5) with 10 N NaOH. The HPLC analysis showed the formation of 2'-AMP, 3'-AMP, 5'-AMP, cyclic (2'-3')-AMP and cyclic (3'-5')-AMP. The main components of the products were 2'- and 3'-AMP, though cyclic (2'-3')-AMP was the main component in the early period of the incubation at pH 9.0. The yields (conversion rate of adenosine to AMPs) were increased almost linearly with the incubation time for 5 months in the case of pH 9.0. The final yields were about 3% (pH 9.0), 6% (pH 9.0, 1 M NaCl), 5% (pH 9.0, 0.01 M $CaCl_2$, 0.01 M $MgCl_2$), 7% (pH 9.0, 0.5 M NaCl, 0.01 M $CaCl_2$, 0.01 M $MgCl_2$), 9% (pH 9.0, 1 M NaCl, 0.01 M $CaCl_2$, 0.01 M $MgCl_2$), 32% (pH 10.5), 43% (pH 11.5), 35% (pH 12.5).

Phosphate is an essential constituent of living organisms and there is no doubt that phosphate might have played important roles in chemical evolution. Various experiments concerning possible prebiotic phosphorylation and dehydration condensation have been performed with phosphates and poly-phosphates (Miller and Orgel, 1974; Yamagata *et al.*, 1979 and 1981). However, no explanations have been given for the occurrence of ortho- and poly-phosphates involved in the experiments in an extensive scale on the primitive Earth. As most phosphate should have occurred in the form of apatite insoluble in water, the concentration of phosphate in the primitive ocean might have been limited to a very low level, as well as in the present ocean. This fact presents a difficulty in chemical evolution as discussed by several authors (Gulick, 1957; Miller and Urey 1959; Miller and Parris, 1964). Miller and Parris (1964) demonstrated the formation of pyrophosphate with the suspension of apatite in aqueous solution of cyanate. But the problem still has not been solved, because calcium and magnesium salts of pyrophosphate are also insoluble. Recently, Griffith (1977) and Griffith *et al.* (1977) proposed a possibility of the formation of tetrametaphosphate via a P_4O_{10} molecule on the primitive Earth. In this report we discuss a possible phosphate cycle on the primitive Earth through the volcanic formation of a P_4O_{10} molecule, and we present experimental results of phosphorylation of adenosine in aqueous solution of phosphorus pentoxide which is converted to tetrametaphosphate by the hydration.

It is well known that phosphorus is easily evolved in the elemental state by igniting a mixture of phosphate rock, silica sand and coke under the exclusion of the air (Ross *et al.*, 1924; Riegel, 1937). If the air is not excluded in the volatilization process, the

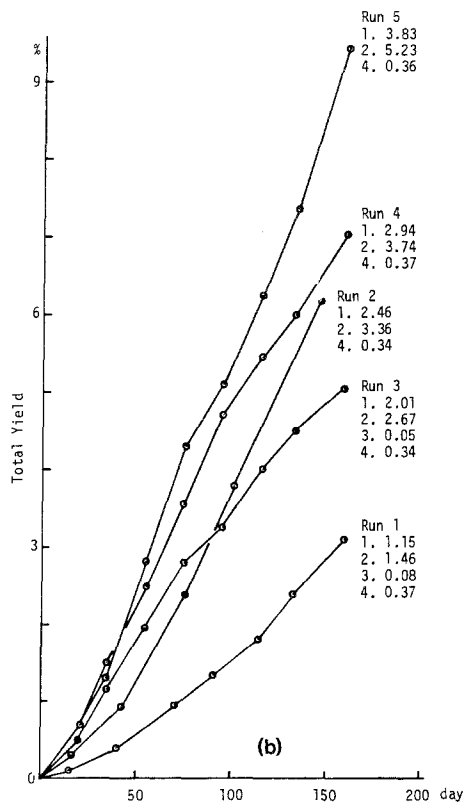
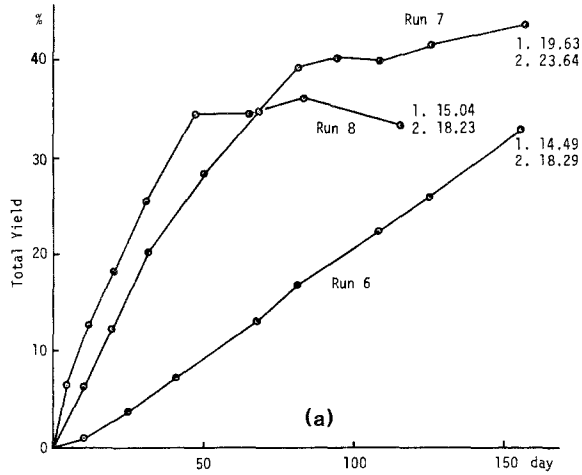


Fig. 1(a) and (b). Dependence of total conversion rate of adenosine to AMP on the incubation time. The values given in the figures show the yield (%) for each AMP at the end of the incubation (1. 2'-AMP, 2. 3'-AMP, 3. 5'-AMP, 4. cyclic (2'-3')-AMP).

The effect of phosphorus pentoxide as a phosphorylating agent has been investigated by the conversion of adenosine to AMP in the aqueous solution. The aqueous solution of phosphorus pentoxide was prepared by adding slowly the oxide into the cold water, stirring vigorously on the ice water bath to avoid the local high temperature, and neutralizing the solution with sodium hydroxide. In the titration, a molar ratio of sodium hydroxide to P_2O_5 was 2.08 which is a value near 2.00 corresponding to the complete conversion of P_2O_5 to tetrametaphosphate (Bell *et al.*, 1952). Each of the experiments was carried out with the aliquot of the solution. The experimental conditions are given in Table I. The solution containing several drops of toluene as the antiseptic was incubated at 37°C with tightly closed bottles. The pH of the solutions was adjusted every day or few days to each fixed value with 10N NaOH, in which the volume of the solution was not changed practically. The analysis was carried out with HPLC [Hitachi Model 638-50; column: Hitachi gel 3013N (anion exchange porous polymer, 5-6 μm) 4 \times 150 mm; column temperature 50°C; elution: computer controlled gradient elution of aqueous solution of ammonium chloride, potassium phosphate, acetonitrile; flow rate 0.5 ml/min; detection UV 260 nm]. In the analysis, a 10 μl or a 20 μl of the sample was applied to the column.

The experimental results are given in Figure 1(a) and 1(b). In all of the runs, the main products were 2'- and 3'-AMP. In the cases of pH lower than 9.0, cyclic (2'-3')-AMP was detected with considerable yield, whereas 5'-AMP and cyclic (3'-5')-AMP with much lower yields (2'-, 3'- and cyclic (2'-3')-AMP were also confirmed on TLC). Several small peaks corresponding to adenosine polyphosphates appeared in all cases, but the peaks did not grow. The addition of CaCl_2 and MgCl_2 into the P_2O_5 solution did not cause any precipitate at the beginning, but the solutions of Run 3, 4, and 5 began to become turbid after 19, 8, and 4 days of the incubation, respectively, and the precipitates increased gradually. The results illustrated in the figures show that Ca^{++} and Mg^{++} ions serve as the catalyst for the phosphorylation reaction, and that sodium chloride is also effective, though at a lower level. At the early period of the incubation, the main component of the products in the Run 1-6 was cyclic (2'-3')-AMP, but the relative yields of 2'- and 3'-AMP were gradually increased through the incubation. This means the formation of 2'- and 3'-AMP via cyclic (2'-3')-AMP.

In conclusion, we propose a possibility of the recycling of phosphate on the primitive Earth. After serving as the effective reagent for phosphorylations, the degraded tetrametaphosphate precipitates to the seabed as the insoluble salts and form the sedimentary rocks, which are again utilized as the raw material of the P_4O_{10} formation when they are brought to a hot place by crustal alteration.

The proposed phosphate recycling might still occur on the present Earth, apart from one by the weathering or via living organisms. It has been reported that phosphate is ejected by volcanic activity (Tageeva, 1942; Oana, 1963). The analysis of the gas from the fumaroles on Showashinzan (a volcano in Japan) shows that phosphate equivalent to about 2.4 mg of phosphorus is contained in 1 m^3 of the gas

(at 0°C, 760 mm Hg) in the case of the specimen collected in sulfuric acid*. This corresponds to about 10^{-4} M of phosphate in the condensed state of the gas, which is 10^2 times larger than in the present ocean. Further, it is noteworthy that in another specimen, in which the gas from the same fumarole was condensed directly, phosphate was detected in a concentration of only about one tenth of the above value. The difference appears to indicate that tetrametaphosphate was largely hydrolyzed in sulfuric acid before the analysis in the former case, whereas little in the latter (the analysis can only detect orthophosphate).

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