SPECIFIC EFFECT OF MAGNESIUM ION ON 2′, 3′-CYCLIC AMP SYNTHESIS FROM ADENOSINE AND TRIMETA PHOSPHATE IN AQUEOUS SOLUTION

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Abstract. Phosphorylation of adenosine by trimetaphosphate was investigated using various catalysts in aqueous solution under mild conditions at pH ~7.0 and at 41 °C. The product was primarily 2′,3′-cyclic AMP together with smaller amounts of ATP. Magnesium ion was found to have a remarkable catalytic effect of approximately one hundred times greater than the other chemicals tested. The mechanism for the specific effect of magnesium ion is discussed.

1. Introduction

Polyphosphates have been shown to be important substances in prebiotic evolution as well as in present organisms. However, the spontaneous appearance of water-soluble polyphosphates on the primitive Earth has been a mystery for a long time (Gulick, 1957; Miller and Urey, 1959; Schwartz, 1971). Recently, we have demonstrated that polyphosphates are produced through volcanic activity (Yamagata *et al.*, 1991). Trimetaphosphate, which is one of the polyphosphates produced from volcanos, has been shown to be the most effective condensing agent among the polyphosphates for peptide formation in aqueous solution (Rabinowitz *et al.*, 1969; Yamanaka *et al.*, 1988). Schwartz (1969) reported that adenosine was phosphorylated in strong alkaline solution by trimetaphosphate to yield 2′- and 3′-AMP, but no reaction was observed in the absence of base. Saffhill (1970) and Etaix and Orgel (1978) investigated more precisely the reaction between nucleosides and trimetaphosphate, and they found the 2′,3′-cyclic nucleotide in the reaction mixtures at lower pH's. Etaix and Orgel (1978) found also nucleoside triphosphate. Tetrametaphosphate, which was assumed to be another natural product from volcanos, was shown to produce mainly 2′- and 3′-AMP by phosphorylation of adenosine in weakly alkaline solution in long term experiments (Yamagata *et al.*, 1982).

We present here results of experiments on the phosphorylation of adenosine with trimetaphosphate in aqueous solution at low temperature and neutral pH. Magnesium ion is shown to be an extremely effective catalyst for the synthesis of 2′,3′-cyclic AMP.

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2. Experimental

To a mixed aqueous solution of adenosine (0.02 M) and trimetaphosphate (0.5 M) were added various potential catalysts. The solutions were allowed to stand at 41 ± 1 °C, and the pH was adjusted with 10 M NaOH solution to keep a constant value around 7.0. The pH of the solutions which contained $MgCl_2$ or $CaCl_2$ changed rapidly. The pH of the stirred solutions was adjusted at ten or fifteen minutes intervals.

The progress of the reaction was monitored by analyzing the solution by HPLC [Hitach Model 638-50; column: Hitachi gel 3013 N (anion exchange porous polymer, 5-6 μm) 4 × 150 mm; column temperature 50 °C; elution: computer controlled gradient elution by aqeuous solutions, A and B [A: aqueous solution of 0.05 M NH_4Cl, 0.0083 M KH_2PO_4, 0.0083 M K_2HPO_4 + acetonitrile {30/2 (V/V)}, B: aqueous solution of 0.6 M NH_4Cl, 0.1 M KH_2PO_4, 0.1 M K_2HPO_4 + acetonitrile {30/2 (V/V)}, 0-14 min, 0% B (100% A); 14-44 min, 0-20% B linear gradiant; 44-80 min, 20-80% B linear gradiant]; flow rate 0.5 ml/min; detection UV 260 nm]. A 10 μl or a 20 μl sample was applied to the column for the analysis.

TABLE I

Adenosine (0.02 M) + Trimetaphosphate (0.5 M) $\xrightarrow{\text{Catalyst}}$ Phosphorylated Adenosine (41 °C, 1 day)

Catalyst	pH	Yield[a] (% × 100) 2′, 3′ cyclic AMP	2′-AMP	3′-AMP	5′-ATP
None	6.9-7.1	3.2	0.1	**0.3**	3.0
$MgCl_2$ (0.2 M)	6.9-7.1	380	0.0	**0.0**	6.4
$CaCl_2$ (0.2 M)	6.9-7.1	15	0.0	**0.0**	0.0
NaCl (4.0 M)	6.8-7.2	1.9	0.0	**0.0**	1.2
NH_4HCO_3 (0.2 M)	6.8-7.2	3.4	0.5	**0.9**	1.9
$NaHCO_3$ (2.0 M)	7.6-8.0	14	6.5	**12**	1.6
K_2HPO_4 (0.2 M)	6.9-7.1	3.5	0.8	**1.7**	2.5
tripolyphosphate (0.2 M)	6.9-7.1	2.7	0.7	**1.5**	1.5
$Ca_3(PO_4)_2$ (3g)	6.7-7.3	3.6	0.2	**0.4**	2.1
$CaCO_3$ (5g)	6.8-7.2	6.0	0.3	**0.6**	0.9
silicagel (6g)	6.9-7.1	2.1	0.3	**1.3**	1.5
Al_2O_3 (8g)	6.8-7.2	4.2	0.0	**0.0**	1.2
imidazole (0.2 M)	6.9-7.1	3.5	1.2	**1.9**	1.2
glycine anhydride (0.1 M)	6.9-7.1	4.9	0.5	**0.9**	1.8
diglycine (0.2 M)	6.9-7.1	2.5	0.7	**1.0**	2.1
triglycine (0.1 M)	6.9-7.1	3.6	0.7	**1.2**	1.0
aspartic acid (0.05 M)	6.8-7.2	3.4	0.3	**0.5**	1.6
D-ribose (0.2 M)	6.9-7.1	3.0	0.3	**0.3**	1.8
urea (0.2 M)	6.8-7.2	3.2	0.5	**0.9**	2.1

[a] Calculated based on adenosine.

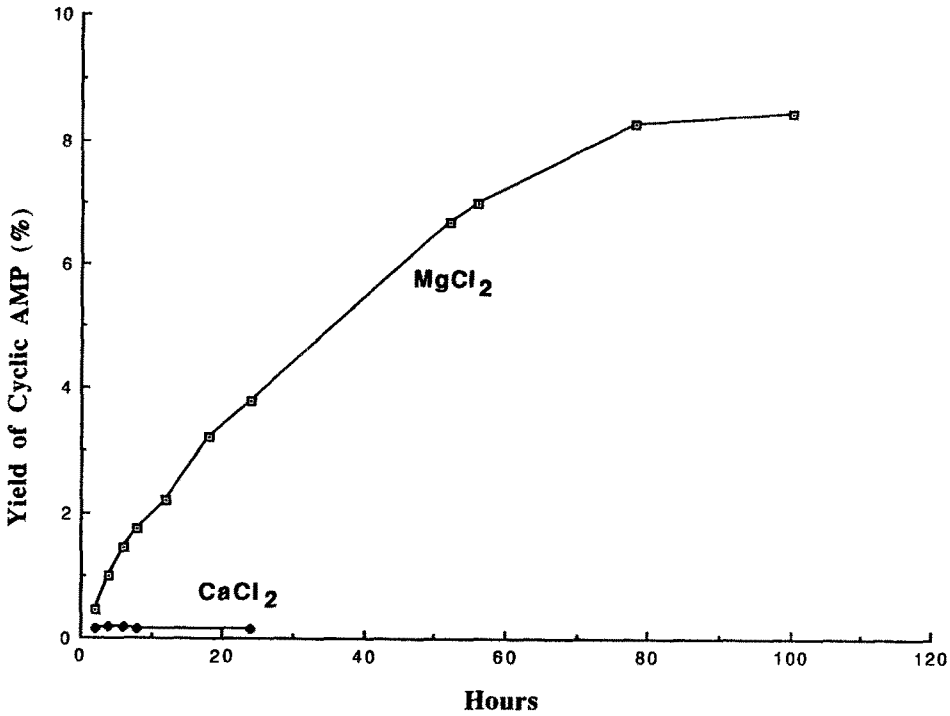

Fig. 1. Comparison of catalytic effect of Mg^{2+} and Ca^{2+} for the 2′,3′-cyclic AMP synthesis.

3. Results and Discussions

2′,3′-Cyclic AMP, its hydrolysis products and ATP are formed in the reaction of adenosine with trimetaphosphate in aqueous solution (Table I). Magnesium ion accelerates the reaction approximately one hundred times more than the other chemicals tested. Figure 1 compares the time course of the reaction by the use of magnesium or calcium ion. The results indicate that calcium ion is much less effective than magnesium ion, though both ions are considered to have similar chemical properties in many respects.

The remarkable acceleration of the reaction by magnesium ion is rationalized by complex formation as shown in Figure 2. Magnesium ion coordinates to trimetaphosphate and adenosine molecules, respectively. The difference between the effects of magnesium and calcium ions found above seems to be due to the difference in ionic radius. Namely, the smaller magnesium ion (radius; 0.65 A) can form the chelating complex with the 2,3-diol group of ribose and attract both molecules more strongly than calcium ion (radius; 0.99 A) does.

Magnesium ion is an essential catalyst for many biochemical reactions, and the primitive ocean may have contained magnesium ion in a high concentration, similarly to the present ocean. It has been shown that magnesium ion has remarkable catalytic

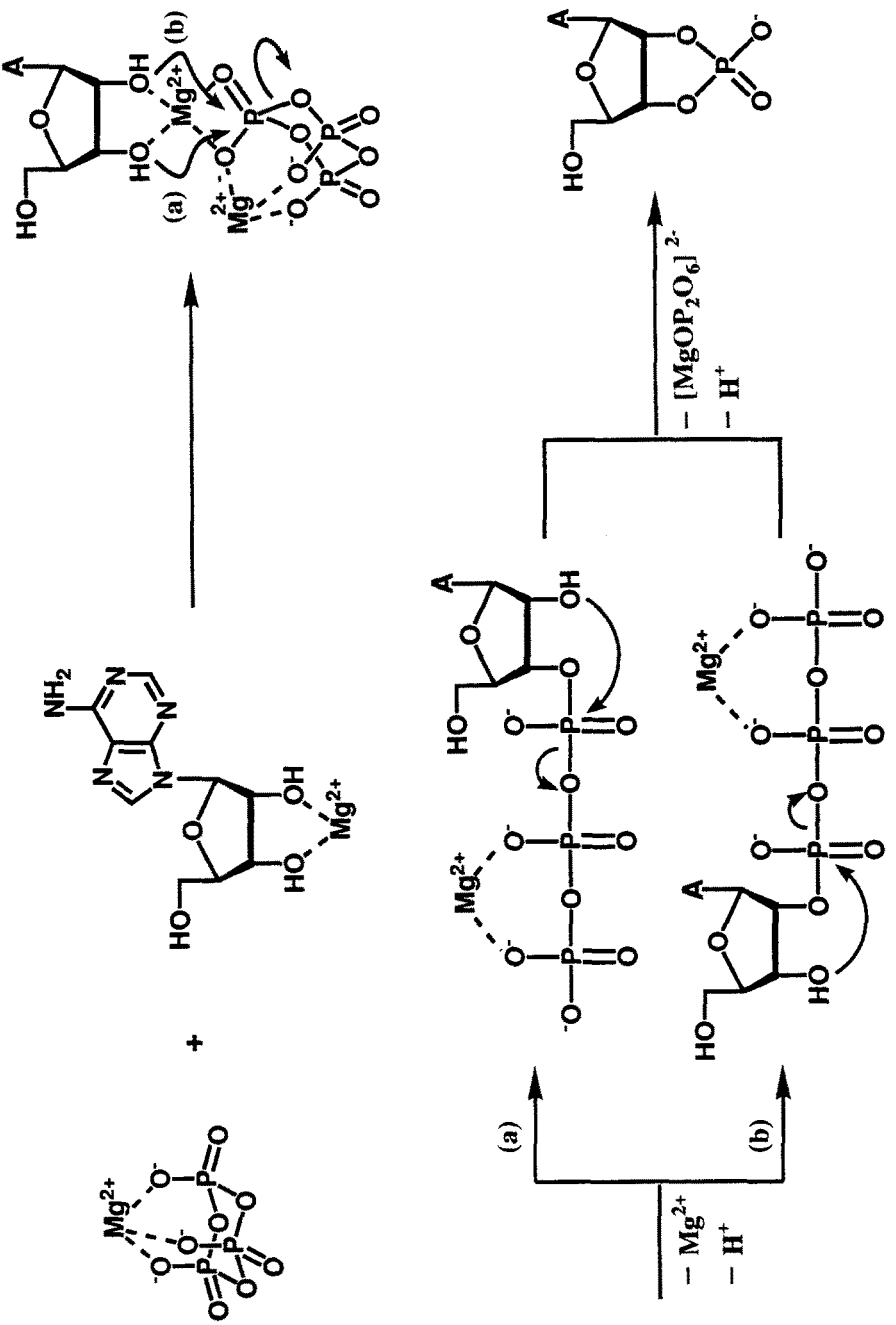

Fig. 2. A possible reaction mechanism for the 2′,3′-cyclic AMP synthesis catalyzed by Mg^{2+}.

activity for prebiotic chemical reactions (Fuller *et al.*, 1972a,b; Handschuh *et al.*, 1973; Lohrmann, 1975, 1977; Lohrmann and Orgel, 1978). However, all of these studies were carried out by heating dried samples. Our present experiments have been carried out in aqueous solution under more natural conditions similar to the biochemical environment, and it was found that the produced 2′,3′-cyclic AMP survived for a relatively long time under the experimental conditions. The present 2′,3′-cyclic AMP synthesis catalyzed by magnesium ion is very interesting, since this molecule has been suggested as a starting monomer for the formation of primitive polyribonucleotides by self-polymerization (Renz *et al.*, 1971; Verlander *et al.*, 1973; Verlander and Orgel, 1974).

Yamagata *et al.* (1982) investigated AMP synthesis from adenosine and tetrametaphosphate, which was spontaneously produced by mild hydrolysis of P_2O_5 (P_4O_{10}) (Bell *et al.*, 1952), in aqueous solution, and found that magnesium and calcium ions had at best a two-fold catalytic effect on the reaction. The comparison between this result and the present one suggests that magnesium ion works specifically as a catalyst for trimetaphosphate, but not with tetrametaphosphate.

Yamanaka *et al.* (1988) reported the condensation of glycine and oligoglycines with trimetaphosphate and tetrametaphosphate in aqueous solutions. The results showed that trimetaphosphate was much more effective, approximately ten times as high as tetrametaphosphate.

In conclusion, the phosphorylation of adenosine by trimetaphosphate was proved to occur mainly at the 2′- and 3′-OH groups of ribose to produce 2′,3′-cyclic AMP as shown in Figure 2. ATP is also produced in lower yield. In alkaline solution, as seen in the case of $NaHCO_3$ in Table I, the cyclic AMP was rapidly hydrolyzed to 2′- and 3′-AMP, similar to the results reported by Schwartz (1969).

Trimetaphosphate might have played an important role to promote prebiotic evolution, and the specific combination of trimetaphosphate and magnesium ion found in the present work is a matter of special interest in prebiotic chemistry. This will be further investigated in future.

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