

PHOSPHORYLATION OF ADENOSINE WITH TRIMETAPHOSPHATE UNDER SIMULATED PREBIOTIC CONDITIONS

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Abstract. The phosphorylation of adenosine with trimetaphosphate in solution, in solid phase and using wet-dry cycles was carried out and it was found that wet-dry cycles were the most efficient. The catalytic effects of some metal ions on the phosphorylation were also studied and it was discovered that Ni(II) is the most effective. The combination of wet-dry cycles (4 cycles) and catalysis by Ni(II) led to an unprecedented high conversion of adenosine to phosphorylated products (30%) near neutral pH. The main phosphorylated products were 2', 3'-cyclic AMP (10.4%) and 5'-ATP (13.0%).

1. Introduction

Phosphorylation of nucleoside is a very important step for the origin of oligonucleotide or nucleic acid. Schwartz (1969) first reported the phosphorylation of adenosine with trimetaphosphate in strong alkaline solution to yield 2'- and 3'-AMP. Saffhill (1970) and Etaix and Orgel (1978) investigated more precisely the reaction between nucleoside and trimetaphosphate, and they found the nucleoside 2',3'-cyclic monophosphate in the reaction mixture at lower pH. Tshako *et al.* (1981, 1984) demonstrated that all ribonucleosides are readily phosphorylated by sodium trimetaphosphate to form their 2'- and 3'-monophosphates in roughly equimolar quantities at high pH and elevated temperature. It is obvious that the above strong alkaline condition (pH 12) might not exist on primitive earth. Yamagata *et al.* (1995) first discovered that the reaction of trimetaphosphate with adenosine could occur in neutral water solution under the catalysis of magnesium ion to afford mainly 2',3'-cyclic AMP. This is a more plausible condition on the primitive earth, but the yield of phosphorylated adenosine is still low (the limit of the yield is about 9%).

In order to increase the conversion of adenosine to phosphorylated products, the reaction was investigated in solution, in solid phase and using wet-dry cycles and it was found that the wet-dry cycles were the most efficient. Based on the wet-dry cycles condition, the catalysts of some metal ions were also studied and nickel ion was found to be more effective than magnesium ion. The total conversion



of adenosine to phosphorylated compounds reached 30% under these conditions. The major products were 2', 3'-cyclic AMP and 5'-ATP, both of which might undergo oligomerization to form oligonucleotide. Furthermore, 5'-ATP, as well known high-energy compound in present life, might act as a condensing agent for the formation of peptide.

2. Experimental

2.1. GENERAL METHODS

2.1.1. *Materials and Instrument*

Adenosine was purchased from Sangon (Shanghai) and all standard materials of isomers of phosphorylated adenosine were purchased from Sigma. Sodium trimetaphosphate was prepared according to the literature (Corbridge, 1985) and confirmed by ^{31}P NMR. All other compounds were analytical reagents. The HPLC analyses ran in Shimadzu LC-9A, using C_{18} reverse column of Zorbax ODS (4.6×25 cm) at room temperature. The eluant was (5mM Na_2HPO_4 + 5mM NaH_2PO_4) / MeOH (100: 25). The flow rate was 0.41 ml/min and the detection UV wavelength was 260 nm. A $5\mu\text{l}$ sample was applied to the column for the analysis.

2.1.2. *Analysis*

Before the HPLC analyses of product mixtures (Table I) were performed, the separation conditions of the mixture of standard compounds of phosphorylated adenosine were carefully optimized and the retention time of each isomer of nucleoside phosphates was determined. As the phosphate substitution of nucleoside doesn't affect the absorption coefficient, the percentage of absorption peak area was approximately used as the percentage of the phosphorylated nucleosides of the product mixture. We repeated the quantitative determinations three times for each standard compound mixture in different concentrations and found that the deviations were less than 0.2%.

2.2. PHOSPHORYLATION OF ADENOSINE UNDER DIFFERENT CONDITIONS

2.2.1. *Reaction in Aqueous Solution*

A 0.4 ml aqueous solution of adenosine (0.02M), sodium trimetaphosphate (0.2M), magnesium chloride (0.1M) and 0.3% formaldehyde (as antiseptic) in a closed phial was allowed to stand in a water-bath oscillator at 37 °C and was frequently adjusted to neutral pH by 10M NaOH. As the reaction proceeded, magnesium phosphate precipitated. The precipitate was filtrated after two weeks and the clear solution was subjected to HPLC analysis. The total conversion of adenosine was 1.3% as shown in Table I. The total conversion of adenosine in our experiment is much lower than that in Yamagata's (1995) experiment due to the lower concentration of magnesium chloride and lower temperature.

2.2.2. *Solid Phase Reaction*

An aqueous (0.4 ml) mixed solution of adenosine (0.02M), sodium trimetaphosphate (0.2M), magnesium chloride (0.1M) was evaporated in vacuum and the obtained white solid was heated at 60 ° in oil bath for 2 weeks in a closed phial in air. The heated solid was washed by 0.4ml distilled water and the filtrate was subjected to HPLC analysis. The total conversion of adenosine was 3.0% as shown in Table I. The conversion in solid phase reaction was higher than in solution, but it must be noted that the reaction temperature was much higher.

2.2.3. *Wet-Dry Cycles Reaction*

A aqueous solution (0.4 ml) of adenosine (0.02M), sodium trimetaphosphate (0.2M) and 0.3% formaldehyde (as antiseptic) in an open tube was adjusted to pH 7 and was allowed to stand at 37 °C in an air-bath oscillator till dryness. Additional 0.4 ml water was added to the dried tube, the mixed solution was adjusted to pH 7 and was allowed to stand at 37 °C in an air-bath oscillator till dryness again (two cycles per week). After four wet-dry cycles (two weeks), 0.4 ml distilled water was added, the precipitate was filtered and the solution was subjected to HPLC analysis. The total conversion of adenosine was 6.9% in the absence of metal ion, as shown in Table I.

2.3. THE EFFECT OF METAL IONS ON THE PHOSPHORYLATION OF ADENOSINE

Aqueous solutions (each tube contains 0.4 ml solution) of adenosine (0.02M), sodium trimetaphosphate (0.2M), metal salt (0.1M) and 0.3% formaldehyde (as antiseptic) were allowed to stand at pH 7 and 37 °C in an air-bath oscillator (without oscillation) to mimic the wet-dry cycles on the primitive earth. When the water evaporated, additional 0.4 ml metal salt (0.1M) solution was added to each tube and adjusted to pH 7 and continued standing in the air-bath oscillator (two cycles per week). After four wet-dry cycles (two weeks), distilled water was added to each tube, the precipitate of each tube was filtered and the solution was subjected to HPLC analysis. The results are listed in Table I.

3. Results and Discussion

The phosphorylation might occur under all three conditions (water solution, solid phase or wet-dry cycles) on the primitive earth, but our experiments showed that the most effective one is the wet-dry cycles. First, in the absence of catalytic metal ion, the reaction solution underwent a concentrating process as the water evaporated, which would enhance the reaction. The reactants in solid phase were also in high concentration, but they were difficult to move and react with each other. The reactants in water solution were constantly in lower concentration and a lower conversion was expected. Second, in the presence of metal ion catalyst, as the

TABLE I
The effects of reaction conditions on the phosphorylation of adenosine

Metal ions	Yields (%) ^a						Total ^d Conv.
	5'-ATP (4.9) ^b	5'-AMP (5.6)	3'-AMP (6.2)	2'-AMP (7.4)	cAMP (10.32)	Ad ^c (11.8)	
Solution reaction							
MgCl ₂	0.6	0.2	0.3	0.1	0.2	98.6	1.3
Solid phase reaction							
MgCl ₂	1.3	0.2	0.5	0.2	0.8	97.0	3.0
Wet-dry cycles reaction							
Without metal ion	3.9	0.0	0.0	0.1	2.6	93.1	6.9
AgNO ₃ ^e	23.7	4.4	1.5	4.8	0.0	65.0	35.0
MgCl ₂	1.6	1.4	4.6	1.8	3.8	85.2	14.8
ZnCl ₂ ^e	0.0	0.2	0.0	0.3	0.2	99.3	0.7
Co(NO ₃) ₂ ^e	0.4	5.3	0.6	2.0	1.2	90.5	9.5
FeCl ₂ ^{f,e}	5.9	1.7	0.7	2.0	0.0	89.5	11.5
FeCl ₃ ^e	0.0	1.1	0.6	0.0	0.0	98.2	1.8
Cu(NO ₃) ₂	Undetectable ^g						
Pb(NO ₃) ₂	Undetectable ^g						
LiCl	3.2	1.3	0.9	3.3	0.5	91.3	8.7
Ni(NO ₃) ₂	13.0	2.8	0.0	4.2	10.4	69.3	30.7

^a Yields are calculated on the peak area of HPLC profile of adenosine related compounds.

^b The data in the parentheses are retention time (minutes) of HPLC analysis. ^c Ad is adenosine. ^d Total conversion of adenosine to phosphorylated products. ^e The total area of peaks was lower than normal (e.g. Mg⁺⁺ catalyzed reaction) due to co-precipitate formation. ^f Saturated FeCl₂ solution. ^g The products and adenosine were totally co-precipitated with metal ions.

phosphorylation took place, the catalytic metal ion was precipitated by phosphate anions produced by the decomposition of trimetaphosphate, and the catalytic ions became less and less in solution. Therefore, the fresh added metal salt at the next cycle could drive the reaction again. We added metal salt solution at each cycle because we think that the catalytic metal salt deposited at the drying process (by solar heating) in a pond would re-dissolve at the wetting process (by raining) on the primitive earth. The magnesium ion might be very abundant on the primitive earth, and it was confirmed that it catalyzed the phosphorylation (14.8%). This result is consistent with the conclusion made by Yamagata (1995). Our experiment further showed that nickel ion is more efficient (30%, twice the yield produced by magnesium ion). It is further expected that the yield of phosphorylation in the presence of nickel ion would be higher if the wet-dry cycles were increased. Although the relative conversion of adenosine in the presence of silver ion is high (35%), the

total area of peaks was very small (one third as in the reaction carried out with magnesium ion) when the same volume of solution sample was subjected to HPLC analysis. This was probably due to the co-precipitation of some of the products with silver phosphate. It is very possible that the ratio of adenosine-related compounds in the co-precipitated part is different from the ratio in solution, therefore, the yields of the phosphorylated products obtained from the solution may not represent the yields of the whole reaction. Furthermore, the precipitated products would not have been available for further reaction. Therefore, it is unlikely that this reaction played a significant role in chemical evolution.

It is notable that the above nickel catalyzed dry-wet cycles reaction not only afforded 2', 3'-cyclic AMP (10.4%) but also 5'-ATP (13.0%) which has proven to be synthesized with difficulty in earlier studies (Gao *et al.*, 2000; Yamagata, 1999). Thus, the alkaline-medium reaction or magnesium catalyzed solution reaction between adenosine and trimetaphosphate didn't give 5'-ATP. Although 2', 3'-Cyclic AMP is very important precursor as it can easily form oligonucleotide (Fox, 1989), 5'-ATP is thought to play a more important role in prebiotic evolution on the primitive Earth. First, it might undergo oligomerization to form oligonucleotide as 5'-polyphosphate activated oligonucleotide was verified to perform template directed ligation (Bartel *et al.*, 1993; Rohatgi *et al.*, 1996; Gao *et al.*, 2000). Second, it is well known that aminoacyladenylate can effectively form peptide in water solution or on a clay surface (Krampitz *et al.*, 1969), and it is suggested that aminoacyladenylate might come from the reaction between amino acid and ATP (Miller and Orgel, 1974).

Thus, the experiments described here of an efficient method to phosphorylate adenosine in high yield near neutral condition, may imply that a nickel-enriched environment could have been of significance for the phosphorylation of nucleoside and the origins of life.

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