SHORT COMMUNICATIONS

Hydrolysis of Adenosine 5'-Triphosphate in the Presence of Various Polyamines

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We have recently investigated the non-enzymatic hydrolysis of adenosine 5'-triphosphate (ATP) with various polyamines in order to find a clue to the ATPase function. We observed that ATP is hydrolyzed effectively and specifically with some amines.

Aqueous solutions of ATP (1×10^{-3}M) containing polyamine hydrochloride (1×10^{-3}M) adjusted at a series of pH values with small quantities of hydrochloric acid or sodium hydroxides, were heated at 60°C for 3 hr. The reaction products, orthophosphate (Pi)[1] and/or pyrophosphate (PPi),[1] were determined by the methods of Martin and Doty[2] and of Tetas and Lowenstein,[3] respectively. For some particularly important data on the amount of Pi and PPi, the results were re-examined on the basis of the amount of nucleotides such as ATP, ADP, and AMP by Cohn-Carter method,[4] showing a satisfactory coincidence with each other. The results of ATP hydrolysis with polyamines, *viz.*, $NH_2CH_2CH_2(NHCH_2CH_2)_nNH_2$ (n=0, 1, 2, 3, and 4) are given in Fig. 1.

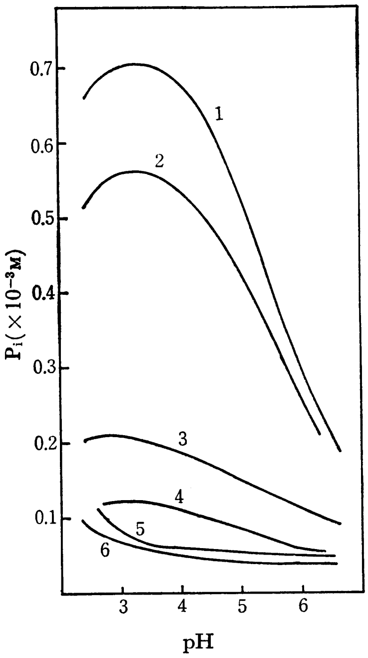

Fig. 1. The results of hydrolysis reactions of ATP with polyamines at various pH values.
1: pentaen (n=4), 2: tetraen (n=3), 3: trien (n=2), 4: dien (n=1), 5: en (n=0), 6: in the absence of amine experimental error: within 2 to 3%

We see that hydrolyses with pentaen,[5] tetraen, trien, or dien show a maximum at pH range 3—4, and the behaviors of pentaen and tetraen show remarkably high activities. The reaction of ATP with pentaen or tetraen also exhibited excellent specificity, producing only ADP and Pi. Figure 2 shows plots of the yields of Pi against ATP-amine ratio. The breaks in the two curves which occur at a 1: 1, amine: ATP system suggest that the intermediates in the hydrolysis reactions consist of equimolecular amounts of ATP and amine. Hydrolysis reactions in the presence of dien(N 2 N 2 N),[6] spmd(N 3 N 4 N), ditn(N 3 N 3 N), trien (N 2 N 2-N 2 N), spmn(N 3 N 4 N 3 N), and dheen (O 2 N-2 N 2 O) were also carried out in water–dioxane (3:2) mixture. The spmd and ditn, and spmn and dheen showed no reaction activities compared with dien and trien, respectively. Of special interest is the fact that no alcohol group in dheen molecule shows activity, and the number of the methylene chains between two nitrogen atoms in a polyamine is extremely important for the hydrolysis reaction.

It may be suggested that there are intermolecular hydrogen bondings between the amino groups of polyamine and the parts of adenine ring, ribose ring and phosphate chain in ATP to activate the phosphate group so that the hydrolytic attack of water molecule becomes easier.

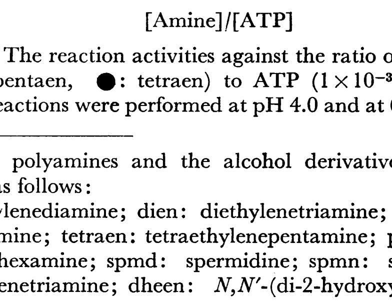

Fig. 2. The reaction activities against the ratio of polyamines. (○: pentaen, ●: tetraen) to ATP (1×10^{-3}M).
The reactions were performed at pH 4.0 and at 60°C for 3 hr.

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[1] These products are shown in the following schemes.
ATP→ADP+Pi ADP: adenosine-5'-diphosphate
ATP→AMP+PPi AMP: adenosine-5'-monophosphate

[2] J. B. Martin and D. M. Doty, *Anal. Chem.*, **21**, 965 (1940).

[3] M. Tetas and J. M. Lowenstein, *Biochem.*, **1963**, 350.

[4] W. E. Cohn and C. E. Carter, *J. Amer. Chem. Soc.*, **72**, 4273 (1950).

[5] The polyamines and the alcohol derivative used are abbreviated as follows:
en: ethylenediamine; dien: diethylenetriamine; trien: triethylenetetramine; tetraen: tetraethylenepentamine; pentaen: pentaethylenehexamine; spmd: spermidine; spmn: spermine; ditn: dipropylenetriamine; dheen: *N,N'*-(di-2-hydroxyethyl)ethylenediamine.

[6] The numerals 2, 3, and 4 symbolize the numbers of methylene chains; notation, for example, N 2 N 2 N and O 2 N 2 N 2 O show $NH_2CH_2CH_2NHCH_2CH_2NH_2$ and $HOCH_2CH_2NHCH_2CH_2NH$-CH_2CH_2OH, respectively.