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Hydrolysis of Adenosine 2',3'-Cyclic Phosphate and Adenylyl(3'-5')adenosine Catalyzed By Alkylenediamines

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Alkylenediamines exhibit remarkable catalysis for the hydrolysis of adenosine 2',3'-cyclic phosphate and adenylyl(3'-5')adenosine. The significant catalysis is ascribed to the intramolecular cooperation between the neutral amine and the protonated amine in the alkylenediamines.

Synthesis of artificial systems for the selective cleavage of nucleic acids (DNAs and RNAs) has been one of the most challenging topics.[1] In most of the cases, metal complexes as catalytic sites were attached to moieties showing specific recognition of nucleic acids. Attempts to prepare artificial nucleases involving organic residues as catalytic sites have not been successful yet, mainly because no organic catalyst shows sufficient activity. Development of highly active organic catalysts is of significance.

Previously [2] the authors showed that alkylenediamines are quite active catalysts for the hydrolysis of activated phosphodiesters bis(nitrophenyl) hydrogenphosphates. There the catalytic activity is largely promoted by the intramolecular cooperation of two amino residues.

The present communication reports that cooperative catalysis by alkylenediamines (H_2N-$(CH_2)_n$-NH_2: N-n-N (n = 1-5)) is satisfactorily applicable to the hydrolysis of phosphodiester linkage in RNAs. Adenosine 2',3'-cyclic phosphate (A>p), an intermediate in the hydrolysis of RNAs,[3] as well as adenylyl(3'-5')adenosine (ApA) is efficiently hydrolyzed by the monocations of the

diamines. Definite requirement of the intramolecular cooperation of two amino groups is evidenced.

Hydrolysis of A>p was carried out at pH 9.0, 50°C unless otherwise noted, and was followed by periodical analysis of the reaction mixture with HPLC.[4,5] All the reactions showed pseudo first-order kinetics. In the catalysis by N-2-N, formation of small amounts of two unidentified byproducts was detected. There the rate of hydrolysis of A>p was evaluated from the rate of appearance of the products 2'- and 3'-phosphates of adenosine. Hydrolysis of ApA was effected at pH 10.0, 50°C in a similar manner.

The alkylenediamines N-n-N (n = 1-5) showed effective catalysis for the hydrolysis of A>p. The rate constant increased linearly with increasing concentration of the diamine up to 0.15 mol dm^{-3} (the largest value examine). The product ratio (adenosine 2'-phosphate to adenosine 3'-phosphate) is almost 1:1. The catalytic rate constants (k_{cat}), determined from the slopes of the linear plots at pH 9.0, 50°C, are listed in Table 1. The catalytic activity steeply increases with the increase in the number n of the methylene chain between the two amino residues from n=1 to n=3. At the value of n larger than 3, however, the activity gradually decreases with increasing n. As a result, the largest catalytic activity is achieved by N-3-N: the cleavage in the presence of 0.15 mol dm^{-3} N-3-N is 7.6 times as fast as that in its absence.

In contrast with the efficient catalysis by the alkylenediamines, ammonia exhibits no measurable catalysis. Thus, two amino residues in one molecule are definitely required for the effective catalysis.

At pH 9.0, the alkylene-diamines N-n-N exist mostly as monocations: the pK_1 and the pK_2 values of N-3-N are 10.6 and 8.6, respectively.[6] The pH-rate constant profile in the pH 8.5-

Table 1. Catalytic rate constants k_{cat} of various amines for the hydrolysis of A>p at pH 9.0, 50°C

Amine	$k_{cat}/10^{-4}$ min^{-1} dm^3 mol^{-1}
N-1-N	2.5
N-2-N	11
N-3-N	27
N-4-N	21
N-5-N	19
ammonia	0.0

11.5 region for the N-3-N-catalyzed reaction is satisfactorily interpreted in terms of the catalysis by the monocation, with a small contribution of the catalysis by the neutral diamine.

Hydrolysis of ApA was also catalyzed by the alkylenediamines. Intramolecular cooperation of two amino groups is also essential here, as clearly shown by no acceleration effect of ammonia. Acceleration of the cleavage by N-3-N (1.0 mol dm^{-3}) was 6.3 fold at pH 10.0, 50°C.

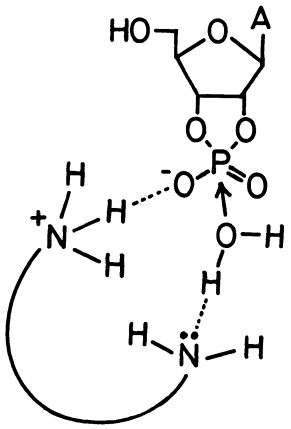

Fig. 1. Proposed mechanism of the catalysis by the monocations of the alkylenediamines for the hydrolysis of A>p.

In the catalysis by the monocation of the alkylenediamine for the hydrolysis of A>p and ApA, the neutral amine and the protonated amine function as general base catalyst and as general acid catalyst, respectively. Overall catalysis is largely promoted by the intramolecular cooperation of the two types of catalysis, as schematically depicted for the hydrolysis of A>p in Fig. 1.

The proposed mechanism is consistent with the mechanism of the diamine-catalyzed hydrolysis of bis(nitrophenyl) hydrogenphosphates.[2] There nucleophilic attack of the neutral amine towards the phosphorus atom is enhanced by the intramolecular assistance of the protonated amine. The mechanism is also supported by the fact that imidazole-catalyzed hydrolysis of poly(uridylic acid) [7] and uridylyl(3'-5')uridine [8] involves the participation of two imidazole molecules, one in acidic form and the other in basic form. These two imidazole molecules intermolecularly cooperate for the catalysis. Acid-base cooperation for the hydrolysis of phosphodiesters was furthermore shown in the catalysis either by cyclodextrins bearing two imidazolyl residues [9] or by 4-imidazoleacetate.[10]

A possibility that the neutral amino residue of the alkylenediamine functions as nucleophilic catalyst rather than general base catalyst is unlikely, since the rate of appearance of the products 2'- and 3'-phosphates of adenosine is exactly

identical with the rate of disappearance of A>p. In the nucleophilic catalysis, phosphoramidate is formed as a rather stable intermediate, and thus a significant difference between the two rates should be observed.

In conclusion, monocations of the alkylenediamines significantly catalyze the hydrolysis of phosphodiester linkage in the ribonucleoside cyclic phosphate and the ribonucleotide dimer. Detailed analysis of the reaction mechanism as well as attempt for the further application of the present finding is currently under way.

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References

1) P. B. Dervan, Science (Washington D. C.), 232, 464 (1986).
2) M. Komiyama and K. Yoshinari, J. Chem. Soc., Chem. Commun., in press.
3) H. Dugas and C. Penney, "Bioorganic Chemistry," Springer-Verlag, New York (1981).
4) M. Komiyama, J. Am. Chem. Soc., 111, 3046 (1989).
5) M. Komiyama and Y. Takeshige, J. Org. Chem., 54, 4936 (1989).
6) A. J. Kirby and W. P. Jencks, J. Am. Chem. Soc., 87, 3209 (1965).
7) R. Breslow, J. B. Doherty, G. Guillot, and C. Lipsey, J. Am. Chem. Soc., 100, 3227 (1978).
8) R. Breslow and M. LaBelle, J. Am. Chem. Soc., 108, 2655 (1986).
9) E. Anslyn and R. Breslow, J. Am. Chem. Soc., 111, 4473 (1989).
10) M. Komiyama, Bull. Chem. Soc. Jpn., in press.

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