Kinetic Analysis of Diamine-Catalyzed RNA Hydrolysis

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The catalysis of various amines for the hydrolysis of RNA has been kinetically investigated, and the catalytic rate constants for each of the ionic states of these amines are determined. Ethylenediamine and 1,3-propanediamine are highly active under the physiological conditions, mainly because they preferentially take the catalytically active monocationic forms. The catalysis of these diamines is further promoted by the intramolecular acid−base cooperation of the neutral amine and the ammonium ion. In contrast, monoamines overwhelmingly exist at pH 7 as the inactive cations. Potential application of the catalysis by the diamines and the related oligoamines is discussed.

Introduction

Recently much interest has been focusing onto nonenzymatic hydrolysis of RNA,[1] and varieties of organic and inorganic catalysts were proposed.[2−5] One of the goals of these studies is the preparation of sequence-selective artificial ribonucleases, which cut a specific RNA at the target site and thus are applicable to molecular biology, therapy, and others.[1c,d] Conjugation of these catalysts with sequence-recognizing moieties was already reported.[1c,d,6] Undoubtedly, molecular design of eminent catalysts for RNA hydrolysis is one of the most important keys for further development of the field.

In a preliminary communication,[2b] we reported that ethylenediamine, diethylenetriamine, and triethylenetetraamine are effective for RNA hydrolysis due to intramolecular cooperation of two amino residues. Furthermore, sequence-selective RNA scission was accomplished by the attachment of oligoamines to synthetic DNA oligomers.[6e,f] The catalyses of the amines are certainly relevant to biological applications.

This paper describes a full account on the RNA hydrolysis by diamines and oligoamines. The catalytically active species are unambiguously characterized, and the catalytic rate constant for each of the species is determined. Dependencies of the catalytic activity on the structure and the basicity of amine are quantitatively clarified. Furthermore, catalytic turnover of the amine is evidenced. The reaction mechanism is proposed in terms of these kinetic results.

Results

Catalysis of Diamines and Oligoamines for RNA Hydrolysis. Table 1 shows the catalytic activities of various amines for the hydrolysis of adenylyl(3′-5′)-adenosine (ApA) at pH 8 and 50 °C. Clearly, the diamines are far more active than the monoamines: the catalytic turnover of the amines is confirmed later. The activity of ethylenediamine (N-2-N) is 13 times as great as that of ethylamine, while *N*,*N*′-dimethylethylenediamine is almost 60 fold more active than *N*-ethylmethylamine. Two amino residues are required for the efficient RNA hydrolysis (the differences in the activity between the diamines and the monoamines are still more remarkable at pH 7 as described below). In a series of unsubstituted alkylenediamines, the activity is in the following order: N-2-N > N-3-N > N-1-N > N-4-N > N-5-N. 1,2-Cyclohexanediamine is more active than the 1,3-counterpart. In the absence of the amines, however, the hydrolysis is virtually nil (the rate constant is evaluated to be 1×10^{-7} min^{-1} from the pH−rate constant profile which is a straight line of slope 1.0 from pH 9.5−13).

Varieties of oligoamines also effectively catalyzed ApA hydrolysis. The rate constants at pH 8 and 50 °C ($[\text{amine}]_0 = 1.0$ M) are 3.6×10^{-5} min^{-1} for diethylenetriamine, 4.6×10^{-5} min^{-1} for triethylenetetraamine, and

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Table 1. Pseudo-First-Order Rate Constants (in 10^{-5} min^{-1}) for the ApA Hydrolysis in the Presence of Amines at pH 8 and 50 °C[a]

catalyst	rate constant	catalyst	rate constant
		Monoamines	
ethylamine	0.57	*N*,*N*,*N*′,*N*′-tetramethylethylenediamine	1.7
diethylamine	0.22	1,3-diaminopropane (N-3-N)	3.8
N-ethylmethylamine	0.21		
		Diamines	
diaminomethane (N-1-N)	2.6	1,4-diaminobutane (N-4-N)	2.0
ethylenediamine (N-2-N)	7.4	1,5-diaminopentane (N-5-N)	1.6
N-methylethylenediamine	7.9	1,2-cyclohexanediamine	6.8
N,*N*′-dimethylethylenediamine	12.9	1,3-cyclohexanediamine	3.9
		none	0.01[b]

[a] $[Amine]_0 = 1.0$ M. [b] Determined from the pH−rate constant profile which is a straight line between pH 9.5 and 13.

5.0×10^{-5} min^{-1} for *N*,*N*′-bis(aminoethyl)-1,3-diaminopropane. Furthermore, poly[A] and poly[U] (as well as other diribonucleotides) were hydrolyzed by these diamines and oligoamines.

In contrast, the corresponding DNA substrates, 2′-deoxyadenylyl(3′-5′)2′-deoxyadenosine and poly[dA], were not hydrolyzed by any of the amines to a measurable extent. It is strongly indicated that the present RNA hydrolysis involves an intramolecular attack by the 2′-hydroxyl group of the ribose.

Totally hydrolytic character of the scission is confirmed by the absence of byproducts such as adenine (formed by the oxidative cleavage of the ribose) and the phosphoramide (due to the nucleophilic attack by the amine toward the phosphorus atom) on HPLC and ^{31}P-NMR: see the Experimental Section. In the hydrolysis of diribonucleotides, the ratio of the 2′-monophosphate of ribonucleoside to the 3′-monophosphate in the products was virtually 1:1, ruling out the possibility of contamination by ribonuclease (the enzyme if any should produce the 3′-monophosphate alone).[7] Divalent metal ions do not participate in the catalysis, since EDTA showed no effect on the hydrolysis rate (note that all the reactions in this paper were achieved in the presence of 2 mM EDTA).

Active Species for the RNA Hydrolysis. The pseudo-first-order rate constant of the ApA hydrolysis (k_{obsd}) by N-2-N and 1,3-propanediamine (N-3-N) at a constant pH linearly increased with the amine concentration. Thus, the apparent second-order catalytic rate constant ($k_{c,appar}$) was determined from the slope of the straight line. Any intermolecular cooperation of two (or more) amine molecules is excluded.

The pH dependencies of $k_{c,appar}$ are depicted in Figure 1. The profiles for both N-2-N (a) and N-3-N (b) are composed of two components, indicating that two ionic states of these diamines (the neutral species and their monocations as shown below) are responsible for the catalysis. Thus, these pH−$k_{c,appar}$ profiles were analyzed in terms of eq 1. Here, k_c's are the partial second-order catalytic rate constants for each of the neutral species, the monocations, and the dications.

$$k_{c,appar} = k_{c,neutral}f_{neutral} + k_{c,monocation}f_{monocation} + k_{c,dication}f_{dication} \quad (1)$$

The molar fraction f of each of the species in the reaction mixtures was calculated by using the pK_a values of the diamines, which were independently measured by a potentiometric titration under the reaction conditions.

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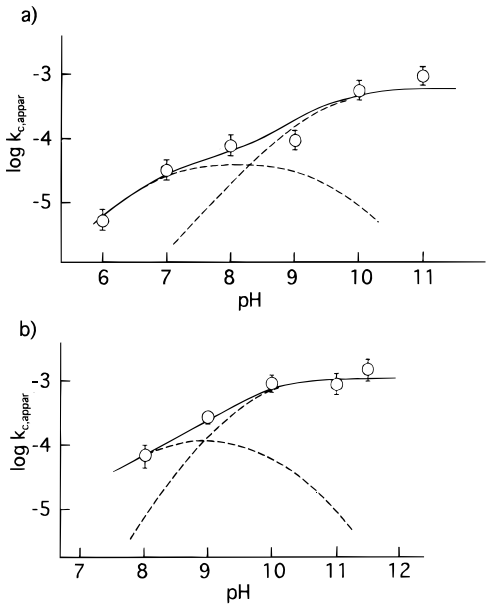

Figure 1. The pH dependencies of $k_{c,appar}$ (in min^{-1} M^{-1}) for the ApA hydrolysis by N-2-N (a) and N-3-N (b) at 50 °C: the solid lines are the theoretical ones calculated by use of the catalytic rate constants and the pK_a values in Table 2. The broken lines show the contribution of each of the species (left: monocation; right, neutral species).

All the experimental points in Figure 1, parts a and b, satisfactorily fit the theoretical lines (the solid ones). The broken lines show the contributions of each of the catalytic species. It is unambiguously confirmed that the profiles cannot be fit in terms of only one catalytic component.

The pH−$k_{c,appar}$ profiles for the diamine-catalyzed hydrolysis of adenosine 2′,3′-cyclic monophosphate (A>p), poly[A], and poly[U] are also bimodal (see the Supporting Information, Figures 1−5). The rate constants for the hydrolysis of poly[A] and poly[U] were evaluated in terms of the decrease of number of the phosphodiester linkages in these polymers: details are described in the Experimental Section.[8]

In the catalysis by ethylamine (NH_2Et), however, the plot of logarithm of $k_{c,appar}$ vs pH was a straight line of slope 1.0 up to pH 9.5, with a gradual saturation at the higher pH. Only the neutral species is catalytically active (the pK_a of NH_2Et is 10.4).

(8) The rates of hydrolysis of poly[A] and poly[U] almost linearly increased with $[N\text{-}2\text{-}N]_0$, indicating that the reactions do not involve significant complexation between the RNA and N-2-N. A deviation due to a polyion complex formation was observed only below pH 7 (see ref 2b).

Table 2. Statistically Corrected Second-Order Catalytic Rate Constants (k_c/q in 10^{-4} min^{-1} M^{-1}) of Amines for RNA Hydrolysis at 50 °C[a,b]

		catalytic rate constant	
substrate	catalyst	monocation	neutral
ApA	N-1-N	0.0 (97)	0.55 (3.1)
	N-2-N	0.44 (61)	2.9 (0.2)
	N-3-N	1.5 (7.4)	7.5 (0.01)
	N-4-N	1.3 (1.6)	8.0 (0.001)
	N-5-N	2.1 (0.8)	15 (0.0003)
	NH_2Et	0.0 (~100)	14 (0.04)
A>p	N-2-N	22 (61)	45 (0.2)
	N-3-N	5.4 (7.4)	110 (0.01)
poly[A]	N-2-N	1.4 (61)	2.6 (0.2)
	NH_2Et	0.0 (~100)	14 (0.04)
poly[U]	N-2-N	1.7 (61)	2.1 (0.2)

[a] The numbers in parentheses are the molar fractions f (in %) of the corresponding species at pH 7. They were evaluated by using the pK_a values of the amines: N-1-N, <3 and 8.5; N-2-N, 6.8 and 9.4; N-3-N, 8.1 and 9.8; N-4-N, 8.8 and 10.1; N-5-N, 9.1 and 10.4; NH_2Et, 10.4. [b] The catalytic rate constants of the neutral diamines are corrected by a statistic factor of 1/2 (see the legend for Figure 2).

Table 3. The Rate Constants at pH 7 and 50 °C for the ApA Hydrolysis by the Amines as well as the Contributions of Each of the Ionic Species[a,b]

	rate constants (10^{-5} min^{-1})		
catalyst	by monocation	by neutral amine	total
N-1-N	0.0	0.34	0.34
N-2-N	2.7	0.12	2.8
N-3-N	1.1	0.02	1.1
N-4-N	0.21	0.00	0.21
N-5-N	0.17	0.00	0.17
NH_2Et	0.0	0.056	0.056

[a] $[Amine]_0 = 1$ M. [b] The rate constants assignable to each of the species (in the two middle columns) were calculated by using the equation: $k_{obsd} = k_c \times [amine]_0 \times$ (molar fraction of the ionic species).

Catalytic Rate Constants of Neutral Diamines and Their Monocations. The catalytic rate constants for each of the ionic species, determined by fitting the pH−rate constant profiles to eq 1 (*vide ante*), are listed in Table 2. The catalytic rate constants for the hydrolysis of A>p, poly[A], and poly[U] are also presented. Here, the values for the neutral diamines are statistically corrected by a factor 1/2, since they have two equivalent amino residues.[9] In all cases, the dicationic diamines are totally inactive ($k_{c,dication} = 0$). At least one of the two amino residues must be neutral for the catalysis, indicating an essential role of a base catalyst for the RNA hydrolysis.

By using the kinetic parameters for the ionic species of the amines and their molar fractions in the mixtures, both of which are shown in Table 2, the rate constants of ApA hydrolysis at pH 7 by these amines are evaluated (Table 3). The contribution of each of the species in the catalysis is also presented there. Apparently, N-2-N and N-3-N exceed the other amines in the activity. It is noteworthy that the catalyses by N-2-N and N-3-N are mostly performed by their monocationic species. Although the neutral diamines are intrinsically more active than the corresponding monocations, their concentrations at pH 7 are too small to make a significant contribution in the catalysis. The same conclusion is obtained for the hydrolysis of A>p, poly[A], and poly[U].

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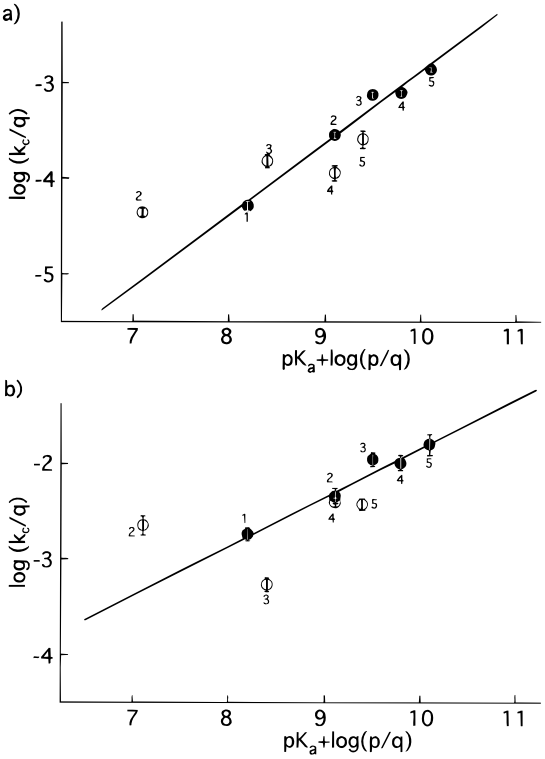

Figure 2. Statistically corrected log−log plots of the catalytic rate constant (in min^{-1} M^{-1}) vs the basicity of the amino residue for the hydrolysis of (a) ApA and (b) A>p at 50 °C: the open circles refer to the monocations, and the closed circles are for the neutral diamines. The kind of amine is designated by the number of the methylene carbon atoms (4 for N-4-N, for example). The statistical corrections were made in k_c and pK_a by using p (the number of equivalent protons which can be transferred from the protonated form of the amine) and q (the number of sites which can accept a proton in the neutral form).[9]

Dependence of the Catalytic Activity on the Basicity of Amino Residue. The closed circles in Figure 2a show the plot of the catalytic rate constant for the neutral species of the diamines for ApA hydrolysis (presented in the fourth column in Table 2) vs the pK_a for the first protonation of the diamine (in footnote a in the table). Here, both k_c and pK_a have been statistically corrected (the details are described in the legend for Figure 2).[9] A fairly linear straight line of a slope 0.75 is obtained. The catalytic activities of these neutral diamines are governed only by the basicities of amino residues.

The relationship between the catalytic rate constant of the monocations (in the third column in Table 2) and the pK_a for the second protonation of the amine is depicted by the open circles. Apparently, the points for the monocations of N-2-N and N-3-N (numbered as 2 and 3) considerably deviate from the straight line (for the neutral diamine) toward the positive side (5 fold for N-2-N and 2 fold for N-3-N).[10] Thus, the catalytic activities of the monocations are greater than are expected simply from the basicities of the neutral amino residues. Intramolecular cooperation of the protonated amino residue with the neutral amino residue is inferred. In contrast, the points for the monocations of N-4-N and N-5-N deviate from the line toward the negative side.

(10) The deviations correspond to the differences in the activities between these monocations and "hypothetical" diamines whose pK_a's for the first protonation are the same with the second protonation of N-2-N and N-3-N.

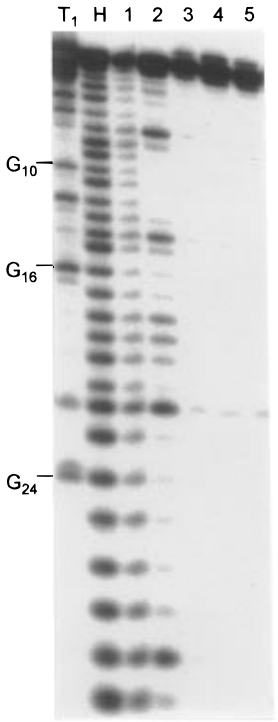

Figure 3. Polyacrylamide gel electrophoresis patterns for the hydrolysis of a 30-mer RNA (^{32}P-labeled at the 3′-end: 10^{-6} M) by N-2-N at pH 8 and 50 °C for 4 h. Lane 1, $[\text{N-2-N}]_0 = 1$ M; lane 2, 0.1 M; lane 3, 0.01 M; lane 4, 0.001 M; lane 5, control. The T_1 and H lanes are for the digestion by ribonuclease T_1 and the alkaline hydrolysis, respectively. The sequence of the RNA is presented in the text. All the cleavage sites are the primary ones.

A similar Brönsted type plot for the hydrolysis of A>p is presented in Figure 2b. In this case, only the point for the monocation of N-2-N positively deviates from the straight line (the slope 0.55), which correlates all the neutral diamine catalysts.[11] The monocationic N-3-N shows a negative deviation from the line.[12]

D_2O Solvent Isotope Effects. Significant D_2O solvent isotope effects were observed for the ApA hydrolysis by the monocation of N-2-N and by its neutral species: $k_c(\text{in } H_2O)/k_c(\text{in } D_2O) = 2.0$ and 1.4, respectively. The catalyses by both of the species undoubtedly involve a proton-transfer in the rate-limiting step.

Scission of RNA Oligomer by N-2-N. Figure 3 depicts the polyacrylamide gel electrophoresis pattern for the N-2-N-catalyzed hydrolysis of a linear 30-mer RNA (5′-GGA GGU CCU GUG UUC GAU CCA CAG AAU UCG-3′) at pH 8 and 50 °C. The pyrimidine−pyrimidine sites are a little bit preferentially cleaved (lane 2: $[\text{N-2-N}]_0 = 0.1$ M).[13] However, the preference is not so strict, since the scission takes place throughout the RNA chain when $[\text{N-2-N}]_0 = 1$ M (lane 1: the secondary scissions are probably involved here). The scission efficiency decreases, as expected, with decrease in the amine concentration and is virtually nil at 1 mM (lane 4).

(11) The intramolecular nucleophilic attack by the 2′-OH in ApA hydrolysis is replaced with the intermolecular one by water in A>p hydrolysis. Thus, the activation entropy term is more critical in A>p hydrolysis. Only N-2-N, which has a minimal conformational freedom, shows the intramolecular cooperation there.

(12) The negative deviations of these monocations from the straight lines are probably ascribed to conformational effects. When the ammonium ions in these molecules electrostatically interact with the negatively charged phosphates of RNA, their neutral amino residues, which function as the general base catalysts, cannot be placed at the most appropriate position for the catalysis.

(13) The preference might be ascribed to smaller steric hindrance.

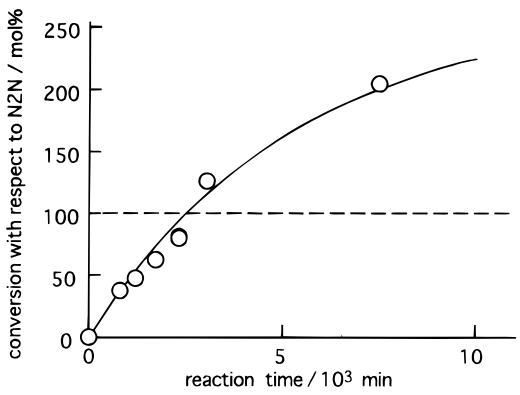

Figure 4. Time−conversion curve for the hydrolysis of ApA by N-2-N at pH 8 and 80 °C: $[\text{ApA}]_0 = 0.27$ M and $[\text{N-2-N}]_0 = 0.1$ M. The open circles show the experimental results, and the solid line is the theoretical one calculated by use of $k_{obsd} = 1.9 \times 10^{-4}$ min^{-1} under the assumption that the reaction shows the pseudo-first-order kinetics until the completion.

Turnover of Oligoamine as Catalyst. When the ApA hydrolysis by N-2-N was achieved under the conditions that $[\text{ApA}]_0 > [\text{N-2-N}]_0$, the conversion of the hydrolysis with respect to N-2-N exceeded 100 mol % (Figure 4). A satisfactory first-order kinetics was observed, confirming that the rate constant was kept constant throughout the reaction (the hydrolysis in the absence of N-2-N is virtually nil). The efficient turnover of N-2-N as catalyst has been concretely evidenced.

Discussion

Factors Governing the Catalytic Activities of Amines. The remarkable catalytic activities of N-2-N and N-3-N for RNA hydrolysis under the physiological conditions (Tables 1−3) are primarily ascribed to the abundance of catalytically active species in the reaction mixtures. Considerable portions of these diamines (61% for N-2-N and 7.4% for N-3-N: Table 2) exist at pH 7 as the catalytic monocations, because of the electrostatic suppression of the second protonation by the positively charged ammonium ions (the corresponding pK_a's are 6.8 and 8.1, respectively). Thus, N-2-N is the most active of all the amines. Furthermore, the intramolecular acid−base cooperation between the neutral amine and the ammonium cation promotes the catalysis of N-2-N and N-3-N by 2−5 fold (see Figure 2). This effect compensates the decrease in the intrinsic catalytic activity (a smaller basicity) of the neutral amino residue, which is caused by the electrostatic effect described above. As the result, the overall catalytic activities ($= k_{c,\text{monocation}} \times f_{\text{monocation}}$) of these monocations are significant.

In contrast, monoamines are only poorly active at pH 7, since they mostly take the protonated forms which are catalytically inactive (the fraction of the active species for NH_2Et is only 0.04%). The diamines N-4-N and N-5-N also dominantly exist as the inactive dications. No activity of the monocation of N-1-N is ascribed to the marginal basicity of its neutral amino residue (the pK_a values of the amines are presented in the footnote a for Table 2).

The catalytic activity of the monocation of N-2-N slightly increases when the nitrogen atoms are methylated: *N*-methylethylenediamine and *N*,*N*′-dimethylethylenediamine are 1.1 and 1.7 times as active as the

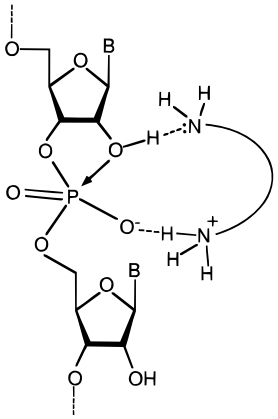

Figure 5. Proposed mechanism for RNA hydrolysis by diamines.

parent N-2-N (Table 1).[14] Thus, versatile moieties can be attached to N-2-N without loss of its catalytic activity.

Catalytic Mechanism of RNA Hydrolysis by Diamines. The proposed mechanism for the catalysis by the monocations of N-2-N and N-3-N in RNA hydrolysis is depicted in Figure 5. The formation of 2′,3′-cyclic monophosphate of the terminal ribonucleotide is rate-limiting in the overall reaction.[7] The neutral amino residue pulls a proton from the 2′-hydroxyl group of the ribose to promote its attack toward the phosphorus atom. The acid catalysis by the protonated amino residue in the same molecule assists the reaction. The cooperation of the two types of catalyses can proceed either simultaneously or in a stepwise manner. The resultant 2′,3′-cyclic monophosphate intermediates are hydrolyzed by a similar cooperation.[11] The mechanism is in accord with the acid−base cooperation of two imidazoles in ribonuclease A,[7] except for the use of two aliphatic amino residues in place of the imidazoles and is also consistent with the successful turnover as the catalyst (Figure 4).

The possibility that the ammonium ions in the monocations of N-2-N and N-3-N function as simple electrostatic catalysts[15] is unlikely in terms of the following results. First, the catalytic rate constant for the ApA hydrolysis by (2-aminoethyl)trimethylammonium ion [$H_2N(CH_2)_2N(CH_3)_3^+$], which has a positive charge as does the monocation of N-2-N but has no acidic proton, is 4.4 times as small as that of the monocationic N-2-N (after the latter is statistically corrected). The difference is exactly identical with the estimated contribution (5-fold promotion) of the intramolecular acid−base cooperation in the monocationic N-2-N (the pK_a of (2-aminoethyl)-trimethylammonium is 6.6, which is virtually identical with that (6.8) for the second protonation of N-2-N). Furthermore, the ammonium ions in the monocations of N-4-N and N-5-N do not assist the catalysis by the neutral amino residues (see Figure 2). Apparently, the length of the alkylene chain between the amino residues has much more a critical effect than is expected for the simple electrostatic catalysis. The intramolecular cooperation is inefficient in the diamines having longer chains (N-4-N and N-5-N), due to the increased conformational freedom of the molecules.[12] Direct nucleophilic attack by the neutral amino residue toward the phosphorus atom is ruled out by (1) a considerable D_2O solvent isotope effect, (2) no detection of the phosphoramide intermediate, and (3) no measurable catalysis of the amines for DNA hydrolysis. The argument is further supported by the fact that the 1:1 combination of (2-aminoethyl)trimethylammonium ion and NH_2Et showed no enhancement in the catalytic activity due to mutual cooperation. Apparently, the acid−base cooperation must be an intramolecular process.

The hydrolysis of ApA and A>p by the neutral diamines, which is dominant in highly alkaline solutions, proceeds via the general base catalysis by one of the two amino residues. The slopes of the Brönsted type plots in Figure 2 (0.75 for ApA hydrolysis and 0.55 for A>p hydrolysis) are consistent with the mechanism.[16]

Conclusion

Diamines and oligoamines, which involve two (or more) amino residues bound by $(CH_2)_2$ or $(CH_2)_3$ chains, efficiently hydrolyze RNA under the physiological conditions. These amines are repeatedly used as catalysts without deterioration. They are simple in structures and are chemically stable so that a wide range of practical applications are promising.

It is noteworthy that the oligoamines have been successfully used as the catalytic sites for sequence-specific artificial ribonucleases.[6e,f] For example, the conjugates of N-2-N and DNA oligomers hydrolyzed the substrate RNA exactly at the target site under mild conditions (pH 8 and 50 °C). There, the concentration of the artificial enzyme (and thus of the N-2-N residue) is only 100 μM. The N-2-N residue is efficiently concentrated at the target phosphodiester linkage due to a proximity effect, when the conjugate and the substrate RNA form a complex. Further increase of the catalytic activity should be plausible if the molecular design is still more precise. The simplicity and stability of the oligoamines are undoubtedly advantageous for the purpose.

Experimental Section

Materials. Dinucleotides, poly[A], poly[U], and poly[dA] were purchased from Sigma or Yamasa. The 30-mer RNA was prepared on a DNA synthesizer using the phosphoramidite method and was ^{32}P-labeled at the 3′-end by using [^{32}P]pCp (from Amersham) and ligase (from Takara). The choice of the sequence is rather arbitrary. Amines, D_2O, DCl (in D_2O), and other reagents were commercially obtained. Water was ion-exchanged and sterilized immediately before use. Throughout the present study, great care was taken to avoid a contamination by natural ribonucleases.

(2-Aminoethyl)trimethylammonium iodide was synthesized from *N*-[(dimethylamino)ethyl]acetamide and methyl iodide in methanol. The acetyl residue, used for the protection of the primary amine in *N*,*N*-dimethylethylenediamine, was removed by 2 N HCl.

Kinetic Measurements. Hydrolyses of dinucleotides (0.1 mM) were performed at 50 °C in pH 8 Tris buffers (50 mM) containing EDTA (2 mM) and were followed by a reversed-phase HPLC: Merck LiChrospher 100 RP18(e) column; eluent, pH 3.4 phosphate buffer containing 4% acetonitrile. The diamine-catalyzed reactions were followed for 3−4 half-lives, although the reactions by monoamines were monitored only for 0.5−1 half-life. The reactions satisfactorily obeyed the

(14) The pK_a values are 6.7 and 9.7 for *N*-methylethylenediamine, and 6.6 and 9.6 for *N*,*N*′-dimethylethylenediamine. The increase of the catalytic activity is probably associated with the enhanced apolar interaction with the substrate, since their pK_a's for the second protonation are rather smaller than the value for N-2-N (6.8).

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pseudo-first-order kinetics. Duplicate or triplicate experiments were carried out, and the rate constants for these reactions coincided with each other within ±3%. For all the amines, the pseudo-first-order rate constant k_{obsd} of the diribonucleotide hydrolysis at a constant pH was proportional to the concentration of amine. Thus the apparent catalytic rate constant ($k_{c,appar}$) was determined from the slope of the straight line of k_{obsd} vs $[amine]_0$. The pK_a values of the amines were measured at 50 °C by potentiometric titration.

The amine-catalyzed hydrolysis of poly[A], poly[U], and poly[dA] (the nucleotide concentration 5 mM) at pH 8 and 50 °C was followed (2−4 half-lives) by ion-exchange HPLC using Toso DEAE-NPR columns: a linear gradient from 0 to 0.4 M $NaClO_4$ at pH 9 in 20 mM Tris buffer. When the polymeric substrates were degraded to smaller fragments, the peaks in the HPLC gradually shifted toward the shorter retention time region (the retention time of the fragmental product monotonously increases with the increase in its molecular weight). The magnitude of the shift of the HPLC peak was converted to the decrease in the number of the phosphodiester linkages in the polymeric substrates, by using the calibration curves (the relationship between the retention time and the degree of polymerization) which were obtained with the use of authentic samples of different degrees of polymerization.[2b]

The hydrolysis products of the 30-mer synthetic RNA (^{32}P-labeled at the 3′-end: 10^{-6} M) were analyzed by a 20% denaturing polyacrylamide gel electrophoresis.

^{31}P-NMR Study on the Reaction Products. At 50 °C, D_2O solutions of ApA (10 mM) were treated with N-2-N (1.0 M). The reaction mixtures were directly subjected to ^{31}P-NMR spectroscopy (a JNM GX-400 FT-NMR spectrometer) with H_3PO_4 (in D_2O) as external standard. At either pD 8 or pD 11, the products were adenosine, its 2′- and 3′-monophosphates, and A>p. No signal assignable to the phosphoramide and other products was detected.[17]

D_2O Solvent Isotope Effects. The isotope effects for the ApA hydrolysis by N-2-N were measured according to eq 1, in which the fractions of the neutral species and the monocation were evaluated by using the pK_a values of N-2-N (7.5 and 10.0 in D_2O). In order to complete D/H exchange, the specimens were prepared by evaporating the sample solutions in D_2O to dryness, followed by the addition of a required amount of D_2O. The pD values were determined by the equation: pD = pH meter reading +0.4.[18]

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Supporting Information Available: pH−rate constant profiles for the hydrolysis of A>p, poly[A], and poly[U] by N-2-N, for the hydrolysis of A>p by N-3-N, and for the hydrolysis of poly[A] by NH_2E (5 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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