RNA hydrolysis by cobalt(III) complexes[1]

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Adenylyl(3′-5′)adenosine (ApA) and uridylyl(3′-5′)uridine (UpU) are hydrolyzed at pH 7.0 and 50 °C by $[Co(N)_4(OH_2)_2]^{3+}$ complexes (N: coordinated nitrogen atom). The pseudo-first-order rate constants for ApA hydrolysis by the triethylenetetramine and the tris(2-aminoethyl)amine complexes (0.1 mol dm^{-3}) are 1.5×10^{-2} h^{-1}, corresponding to 10^5-fold acceleration. The Co^{III} complexes are also active for the hydrolysis of 2′,3′-cyclic monophosphates of ribonucleotides. The pH–rate constant profile for ApA hydrolysis is bell-shaped with a maximum around pH 7, and a notable D_2O solvent isotope effect (2.0) is observed. It is proposed that the coordination water molecules on the Co^{III} ions promote (as general acid catalysts) the departure of the alkoxide ion of 5′-OH from the phosphorus atom.

Introduction

There has been growing interest in the non-enzymatic hydrolysis of RNA, and many catalysts (both organic and inorganic) have been reported.[2–10] Some of them are active enough to hydrolyze RNA under physiological conditions, and are promising in biotechnology, as therapeutics and in other areas. However, detailed information on the catalytic mechanisms is required to design still more active and useful catalysts, but is not yet abundant. For example, the precise roles of acid catalysis and base catalysis in the reactions have not been satisfactorily understood.

Previously, the catalysis of Co^{III} complexes for the hydrolysis of various phosphate esters has been widely and precisely studied.[11–18] Stabilities of these metal complexes in reaction mixtures are certainly advantageous in the characterization of catalytic mechanisms. Concrete evidence for two types of reaction pathways has been found: (1) intracomplex nucleophilic attack by Co^{III}-bound hydroxide ion towards the phosphoesters which are coordinated to the same Co^{III} ion, and (2) intermolecular attack by hydroxide ion and/or water in the media towards the phosphoesters which are activated by coordination to Co^{III} complexes. RNA hydrolysis involves (in most cases) intramolecular nucleophilic attack by the 2′-OH of a ribose toward the phosphorus atom, followed by the departure of the alkoxide ion of 5′-OH from the resulting intermediate. Thus either general acid catalysis or general base catalysis should be required.

We report here the hydrolysis of adenylyl(3′-5′)adenosine (ApA) and uridylyl(3′-5′)uridine (UpU) by diaquatetraazacobalt(III) complexes $[Co(N)_4(OH_2)_2]^{3+}$ (N: coordinated nitrogen atom). 2′,3′-Cyclic monophosphates of ribonucleosides (the intermediates in RNA hydrolysis) are also hydrolyzed. Furthermore, phenyl ester of adenosine 3′-monophosphate (ApΦ), which has a better leaving group than ApA, is used as a probe to assign the rate-limiting step in RNA hydrolysis. A novel mechanism involving general acid catalysis by Co^{III}-bound water is proposed on the basis of a variety of kinetic evidence.

Results

RNA hydrolysis by Co^{III} complexes

At pH 7.0 and 50 °C, $[Co(trien)(OH_2)_2]^{3+}$, $[Co(tren)(OH_2)_2]^{3+}$, $[Co(trpn)(OH_2)_2]^{3+}$, $[Co(en)_2(OH_2)_2]^{3+}$, $[Co(tme)_2(OH_2)_2]^{3+}$ and $[Co(tn)_2(OH_2)_2]^{3+}$ complexes hydrolyzed ApA, as clearly shown by reversed-phase HPLC. Here, trien, tren, trpn, en, tme and tn represent triethylenetetramine, tris(2-aminoethyl)amine, tris(3-aminopropyl)amine, ethylenediamine, 1,1,2,2-tetramethylethylenediamine and 1,3-diaminopropane, respectively. The products were adenosine (Ado) and its 2′- and 3′-monophosphates (A2′p and A3′p). A small amount of the 2′,3′-cyclic monophosphate of adenosine (A>p) was accumulated in the reaction mixtures. The ratio of A3′p to A2′p in the product was almost 1:1, indicating that the P–O(2′) and the P–O(3′) bonds in A>p are cleaved without a specific preference.

Table 1 Pseudo-first-order rate constants for the hydrolysis of ApA, A>p and ApΦ by various Co^{III} complexes at pH 7.0

	Rate constant/10^{-3} h^{-1}		
Ligand	ApA[a] (at 50 °C)	A>p[b] (at 30 °C)	ApΦ[a] (at 50 °C)
trien	15	1700	1800
tren	15	110	1600
trpn	5.6	5600	—
$(en)_2$	4.7	30	900
$(tme)_2$	3.3	1300	—
$(tn)_2$	1.3	1900	—
none	2×10^{-4}[c]	2×10^{-3}[c]	40

[a] $[Co^{III}\ complex]_0 = 0.1$ mol dm^{-3}. [b] $[Co^{III}\ complex]_0 = 0.01$ mol dm^{-3}. [c] Estimated from the pH–rate constant profile which is a straight line of slope 1.0 in the pH 8–13 region.

Adenine, which should be released if the ribose were oxidatively cleaved, was not formed. No isomerization of ApA to adenylyl(2′-5′)adenosine was seen. 2′-Deoxyadenylyl(3′-5′)2′-deoxyadenosine [d(ApA)] was not hydrolyzed by any of the Co^{III} complexes. It is conclusive therefore that the present RNA hydrolysis proceeds *via* intramolecular nucleophilic attack by the 2′-OH of ribose towards the phosphodiester linkage.

The pseudo-first-order rate constants for ApA hydrolysis by $[Co(trien)(OH_2)_2]^{3+}$ and $[Co(tren)(OH_2)_2]^{3+}$ (0.1 mol dm^{-3}) are 1.5×10^{-2} h^{-1} (see Table 1). This corresponds to a nearly 10^5-fold acceleration with respect to the uncatalyzed reaction. The catalytic activity is only slightly dependent on the structure of ligand [the trien and the tren complexes, which are the most active, are only ten times more active than the least active $(tn)_2$ complex]. For all the Co^{III} complexes, the rate of UpU hydrolysis was virtually the same as the value for ApA hydrolysis; *e.g.* the rate constant for UpU hydrolysis by $[Co(trien)(OH_2)_2]^{3+}$ (0.1 mol dm^{-3}) is 1.2×10^{-2} h^{-1}. Any specific interactions of the Co^{III} complexes with the nucleic acid bases in dinucleotides are unlikely.

In contrast with the significant catalysis by these $[Co(N)_4$-

$(OH_2)_2]^{3+}$ complexes, $[Co(tetren)(OH_2)]^{3+}$ (tetren: tetraethylenepentamine) and $[Co(en)_3]^{3+}$ showed no measurable catalysis for ApA hydrolysis. The Co^{III} complex of 1,4,8,11-tetraazacyclotetradecane, which favorably takes the *trans* form,[19] was also inactive.[11a] In order to hydrolyze RNA efficiently, two water molecules must be coordinated to Co^{III} ion in the *cis* position.[20]

Hydrolysis of 2′,3′-cyclic monophosphates of ribonucleosides by Co^{III} complexes

The Co^{III} complexes also promote the hydrolysis of A>p (Table 1). The acceleration by $[Co(trpn)(OH_2)_2]^{3+}$ (0.01 mol dm^{-3}) is more than 10^6-fold. Significantly, the dependence of the activities of Co^{III} complexes on ligand structure for A>p hydrolysis is far more drastic than is the activity dependence for dinucleotide hydrolysis [the most active trpn complex is 190 times as active as the $(en)_2$ complex]. 2′,3′-Cyclic monophosphates of guanosine, uridine and cytidine were hydrolyzed at almost the same rates as A>p.

For all the Co^{III} complexes, A>p hydrolysis is far faster than ApA hydrolysis, even at a lower temperature (30 *vs.* 50 °C) and with less Co^{III} complex (0.01 *vs.* 0.1 mol dm^{-3}). Apparently, formation of the 2′,3′-cyclic monophosphate of ribonucleotide, rather than its hydrolysis to monophosphate, is rate-limiting in Co^{III} complex-induced RNA hydrolysis.

Kinetic analysis of RNA hydrolysis by Co^{III} complex

The rate of $[Co(trien)(OH_2)_2]^{3+}$-induced ApA hydrolysis linearly increased with increasing concentration of the Co^{III} complex. Only one Co^{III} complex participates in the catalysis.

The pH–rate constant profile is bell-shaped and shows a maximum around pH 7 [Fig. 1(*a*)]. All the experimental points fit fairly the theoretical line (the solid one) calculated under the assumption that the combination of trivalent $[Co(trien)(OH_2)_2]^{3+}$ species and hydroxide ion is responsible for the catalysis. The concentrations of three types of Co^{III} species in the solutions [see eqn. (1)] were evaluated by using the equilibrium constants K_{a1} and K_{a2} ($10^{-5.9}$ and $10^{-8.1}$, respectively).

$$[Co(trien)(OH_2)_2]^{3+} \overset{K_{a1}}{\longleftrightarrow} [Co(trien)(OH_2)(OH)]^{2+} \overset{K_{a2}}{\longleftrightarrow} [Co(trien)(OH)_2]^{+1} \quad (1)$$

D_2O solvent isotope effect

$[Co(trien)(OH_2)_2]^{3+}$-induced hydrolysis of ApA was accompanied by a considerable D_2O solvent isotope effect. The value of k_1k_2/k_{-1} in D_2O is 2.0 ± 0.2 times as small as that in H_2O [see eqn. (3) below]. Here, the pD–rate constant profile in D_2O was analyzed by using the corresponding K_{a1} and K_{a2} values ($10^{-6.2}$ and $10^{-8.4}$), which were determined in D_2O by potentiometric titration. The present catalysis by the Co^{III} complex involves a proton-transfer in the rate-limiting step.

ApΦ hydrolysis by Co^{III} complexes

$[Co(N)_4(H_2O)_2]^{3+}$ complexes also accelerated the hydrolysis of ApΦ (the fourth column in Table 1). The products were A>p and phenol (the A>p was then more slowly hydrolyzed to A2′p and A3′p). The reaction proceeds *via* intramolecular attack by the 2′-OH of ribose as does RNA hydrolysis. Significantly, the catalytic activities of Co^{III} complexes for ApΦ hydrolysis (to A>p and phenol) are more than 10^3-fold smaller than those for ApA hydrolysis. For example, the accelerations by the trien and the tren complexes (0.1 mol dm^{-3}) are only 45- and 40-fold respectively, whereas these complexes accelerate ApA hydrolysis by 7.5×10^4-fold.

The pH–rate constant profile is presented in Fig. 1(*b*). In contrast with the bell-shaped profile for ApA hydrolysis [Fig. 1(*a*)], the rate constant monotonically increases with pH. The slope (0.3–0.6) is smaller than 1 throughout the pH range investigated. No measurable D_2O solvent isotope effects were observed [on the values of k_3 and k_4 in eqn. (4)]. In the absence of the Co^{III} complexes, however, the rate of ApΦ hydrolysis is approximately proportional to pH with a slope 1.

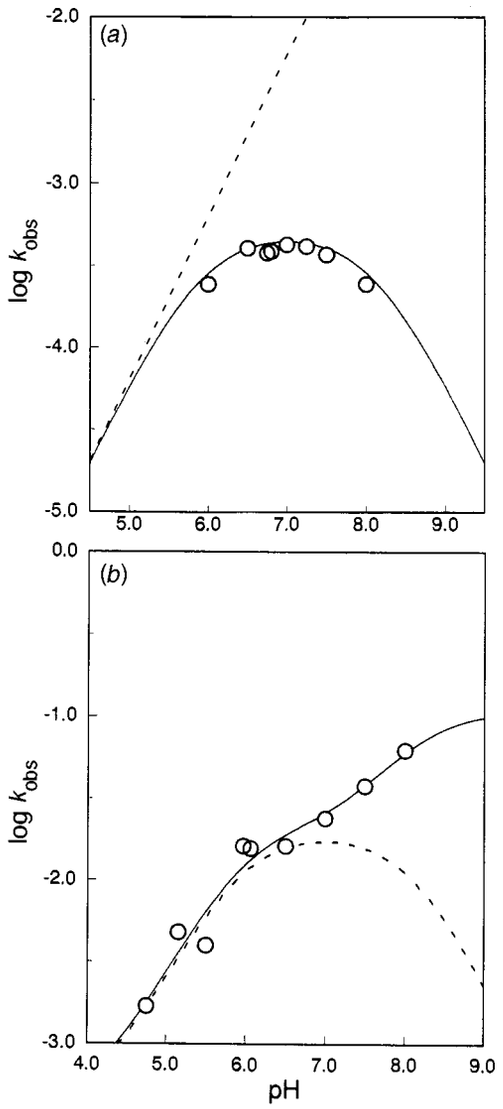

Fig. 1 pH–rate constant profiles for $[Co(trien)(H_2O)_2]^{3+}$-induced hydrolysis of ApA (*a*) and ApΦ (*b*) at 50 °C; [ApA or ApΦ]$_0 = 10^{-4}$ and [Co^{III}]$_0 = 10^{-1}$ mol dm^{-3}. The solid line in (*a*) is the theoretical one calculated by using eqn. (3) ($k_1k_2/k_{-1} = 6 \times 10^5$ min^{-1} mol^{-2} dm^6), whereas the solid line in (*b*) is obtained from eqn. (4) ($k_3 = 9 \times 10^5$ min^{-1} mol^{-2} dm^6 and $k_4 = 3 \times 10^7$ min^{-1} mol^{-2} dm^6).[28] The K_a values for the hydration water on the Co^{III} complex are $10^{-5.9}$ and $10^{-8.1}$.

The dotted lines are obtained under the following hypothetical conditions. (1) Both the $[Co(trien)(H_2O)_2]^{3+}/OH^-$ and the $[Co(trien)(H_2O)(HO)]^{2+}/OH^-$ combinations are active for RNA hydrolysis [in (*a*)], and (2) only $[Co(trien)(H_2O)_2]^{3+}$ or the $[Co(trien)(H_2O)_2]^{3+}/OH^-$ combination is active for ApΦ hydrolysis [in (*b*)]. The parameters presented above are also used. See text for detail.

Discussion

Assignment of the step which is promoted by Co^{III} complexes

In the absence of Co^{III} complexes, ApΦ is hydrolyzed 10^5-fold more promptly than is ApA (Table 1). Replacement of the adenosine in the 3′-side of ApA by phenol, which is a better leaving group (pK_a 9.8), enormously promotes the hydrolysis of the phosphodiester linkage therein. This result is consistent with the recent findings by McLaughlin *et al.* and Taira *et al.*[21] that the substitution of the 5′-oxygen in RNA by sulfur atom activates the alkaline hydrolysis of the corresponding phosphodiester linkage by 10^4–10^6 fold. It has been proposed that

the rate-limiting step in alkaline hydrolysis of RNA is the departure of the alkoxide ion of 5′-OH from the phosphorus atom, rather than the intramolecular nucleophilic attack by the 2′-OH of ribose towards the phosphorus atom;[21b,22] the pK_a of a thiol (~10.5) is more than five units smaller than that of an alcohol.[23] The 5′O→S substitution does not seem to enhance the intramolecular nucleophilic attack by the 2′-OH, since the sulfur atom is intrinsically less electronegative than the oxygen atom. With total consistency the hydrolysis is not significantly accelerated when non-bridging oxygen (either in the *R* position or in the *S* position) in the phosphodiester linkage is substituted by a sulfur atom.[24,25] Thus, the catalysis by Co^{III} complexes for RNA hydrolysis should be ascribed, at least primarily, to the promotion of rate-limiting removal of the alkoxide ion of 5′-OH from the phosphorus atom.

Proposed mechanism of RNA hydrolysis by Co^{III} complexes

Based on the kinetic evidences, the reaction scheme for Co^{III} complex-induced hydrolysis of ApA is proposed as eqn. (2)

$$[Co(N)_4(OH_2)_2]^{3+} + ApA \overset{K,\ -H_2O}{\rightleftharpoons} [Co(N)_4(OH_2)(ApA)]^{2+} \overset{k_1(OH^-)}{\underset{k_{-1}}{\rightleftharpoons}} [Co(N)_4(OH_2)(PI)]^{1+} \xrightarrow{k_2} A{>}p + Ado + [Co(N)_4(OH_2)(OH)]^{2+} \quad (2)$$

where PI is the pentacoordinated intermediate formed by intramolecular attack by the 2′-OH on the phosphorus atom. First, the phosphate of ApA is coordinated to $[Co(N)_4(OH_2)_2]^{3+}$, by ligand exchange with one of the two coordination water molecules on the Co^{III} (this anation step is too fast to be rate-limiting). Electrophilicity of the phosphate is enhanced by 20–50-fold here. Then the 2′-OH of ribose attacks the phosphate as an intramolecular nucleophile, with the aid of hydroxide ion. The hydroxide ion either acts as a general base catalyst to activate the 2′-OH or simply shifts the equilibrium (2′-OH→2′-O^- + H^+) towards the dissociation side. In the departure of the alkoxide ion of 5′-OH from the intermediate, which is rate-limiting in the whole reaction, the coordination water on the Co^{III} ion functions as a general acid catalyst (the notable D_2O solvent isotope effect is associated with this process). The mechanism for the acid catalysis is schematically depicted in Fig. 2(*a*).

As $k_{-1} \gg k_2$ (the decomposition of the intermediate is rate-limiting), the observed rate constant k_{obs} for ApA hydrolysis is expressed by eqn. (3), where $[Co(N)_4(OH_2)_2]^{3+}$ is the concen-

$$k_{obs} = k_1k_2[OH^-]/k_{-1} \times K[Co(N)_4(OH_2)_2]^{3+} \quad (3)$$

tration of the corresponding trivalent species in the solutions (and not the total concentration of the Co^{III} complex). The equilibrium constant for the complex formation between the Co^{III} complex and ApA is represented by *K* [see eqn. (2)]. Eqn. (3) satisfactorily explains all the experimental results [the solid line in Fig. 1(*a*) is based on this reaction scheme].[26]

The mechanism in which the $[Co(trien)(OH_2)(OH)]^{2+}$ species catalyzes the reaction without the assistance of hydroxide ion is unlikely, although it is not ruled out simply in terms of the pH–rate constant profile. If it were really the case, the catalysis for ApA hydrolysis, which takes place in $[Co(trien)(OH)(ApA)]^{1+}$, should be marginal, since the Co^{III}-bound hydroxide ion is poor as an acid catalyst and thus hardly promotes the rate-limiting departure of the 5′-leaving group from the phosphorus atom. Only the $[Co(trien)(OH_2)_2]^{3+}$ species, in which two water molecules (one for the anation and another for the acid catalysis) are bound to the Co^{III} ion, is effective for the purpose.[27] The experimental points in Fig. 1(*a*) do not fit the theoretical line (the dotted one) for the mechanism in which both the

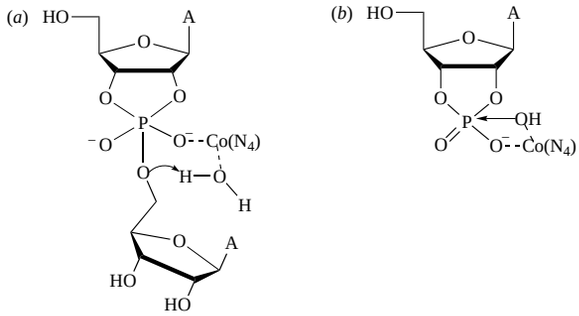

Fig. 2 Proposed mechanisms for $[Co(N)_4(H_2O)_2]^{3+}$ complex-induced hydrolysis of ApA (*a*) and A>p (*b*)

$[Co(trien)(OH_2)_2]^{3+}/OH^-$ combination and the $[Co(trien)(OH_2)(OH)]^{2+}/OH^-$ combination are catalytically active.[29]

Hydrolysis of A>p by the Co^{III} complexes presumably proceeds *via* intracomplex nucleophilic attack by the metal-bound hydroxide ion on the phosphorus atom [Fig. 2(*b*)]. The dependency of the activities of Co^{III} complexes on ligand structure [trpn > trien > tren > $(en)_2$] is identical with that for Co^{III} complex-induced hydrolysis of diaryl phosphates,[11–17] in which the intracomplex nucleophilic attack is firmly established.[13a,b] Consistently, there is no explicit correlation between the activities of Co^{III} complexes for ApA hydrolysis (to A>p and Ado) and those for A>p hydrolysis. In the latter reaction, the magnitude of strain in the four-membered ring, formed on the intracomplex attack by the Co^{III}-bound hydroxide ion, is crucial, resulting in a drastic dependence of the reaction rate on ligand structure.[13b] Such a steric restraint is absent in the general acid catalysis for the ApA hydrolysis.

Hydrolysis of ApΦ

The proposed mechanism of Co^{III} complex-induced RNA hydrolysis is supported by the fact that its pH–rate constant profile [Fig. 1(*a*)] is entirely different in shape from that for ApΦ hydrolysis (*b*). The rate-limiting steps differ from each other. It is unlikely that the decomposition of the intermediate is rate-limiting only for ApΦ which has a better leaving group and is hydrolyzed much more promptly. The rate-limiting decomposition of the pentacoordinated intermediate in the RNA hydrolysis has been furthermore substantiated.

In Co^{III} complex-induced ApΦ hydrolysis, the leaving group (the phenolate) is so good that no acid catalyst is required to remove it from the phosphorus atom. Thus, the $[Co(trien)(OH_2)(OH)]^{2+}$ species {as well as the $[Co(trien)(OH_2)_2]^{3+}$ species} is potent for the catalysis, although it has no coordination water which is applicable to the acid catalysis (when the substrate is bound to the Co^{III}, the metal-bound water is removed in exchange). The solid line in Fig. 1(*b*) is based on these assumptions. The results are never interpreted in terms of the mechanism involving either $[Co(trien)(OH_2)(OH)]^{2+}$ or the $[Co(trien)(OH_2)_2]^{3+}/OH^-$ combination as the sole catalytic species (the dotted line is for these hypothetical mechanisms). The k_3 and k_4 terms in eqn. (4) refer to either general base

$$k_{obs} = k_3[OH^-] \times K'[Co(trien)(OH_2)(OH)]^{2+} + k_4[OH^-] \times K''[Co(trien)(OH_2)_2]^{3+} \quad (4)$$

catalysis by hydroxide ion or the hydroxide ion-promoted dissociation of the 2′-OH to the alkoxide ion in equilibrium, as does the k_1 step in eqn. (2). Here, K' and K'' are the equilibrium constants for the complex formation between the corresponding Co^{III} complex species and ApΦ.

In RNA hydrolysis, however, the acid catalysis by the Co^{III}-bound water is inevitable to remove the alkoxide ion of 5′-OH (a poor leaving group) from the phosphorus atom. All these arguments have been substantiated by the following three

results. (1) Significant D_2O solvent isotope effects are observed in ApA hydrolysis by Co^{III} complexes, but not in ApΦ hydrolysis. (2) The catalytic effects of the Co^{III} complexes for RNA hydrolysis are far greater (1000-fold or more) than those for ApΦ hydrolysis (Table 1). (3) The magnitude of acceleration by the Co^{III} complex for ApΦ hydrolysis (20–45 fold) is mostly interpreted in terms of electrostatic increase of the electrophilicity of the phosphate by Co^{III} complexes.[30]

Conclusion

The phosphodiester linkages in RNA are hydrolyzed by diaquatetraazacobalt(III) complexes, and the reaction mechanism has been clarified. The main role of the complexes is to promote the rate-limiting departure of the alkoxide ion of 5′-OH from the phosphorus atom through general acid catalysis by their coordination water. If the departure is sufficiently accelerated by the general acid catalysis and the formation of intermediate has become (partially) rate-limiting, then general base catalysts should be of importance. Thus, dinuclear and trinuclear metal complexes, in which two or three metal ions (or their coordination water) share the roles as acid catalyst and base catalyst, are remarkably active.[31] The present finding of general acid catalysis by Co^{III} complexes should provide fundamental information for the design of active catalysts for RNA hydrolysis and also greatly widen the scope of the reactions catalyzed by these complexes.

Experimental

Materials

ApΦ was prepared from A>p and phenol, as described previously.[32] ApA, UpU, d(ApA), 2′,3′-cyclic monophosphates of ribonucleosides and adenylyl(2′-5′)adenosine were purchased from Seikagaku Kogyo. Tris(3-aminopropyl)amine (trpn) was synthesized by catalytic hydrogenation of the addition product of acrylonitrile and ammonia.[33,34] 1,1,2,2-Tetramethylethylenediamine (tme) was prepared by hydrogenation of 2,3-dimethyl-2,3-dinitrobutane.[34] Other amine ligands were obtained from Nacalai. $[Co(N)_4Cl_2]^+$ complexes as well as $[Co(N)_5Cl]^{2+}$ and $[Co(N)_6]^{3+}$ complexes were synthesized by methods in the literature.[35,36] D_2O (99.9 atom% D) was purchased from Aldrich.

Hydrolysis by Co^{III} complexes

Hydrolysis of ApA was achieved as follows, mostly according to the procedure employed by Chin *et al*. for the Co^{III} complex-catalyzed hydrolysis of diaryl phosphates.[13b] To an aqueous solution of $[Co(N)_4Cl_2]^+$ complex (0.1 mol dm^{-3} unless otherwise noted), 1.5 equiv. of NaOH was added. The mixture was incubated for 20 min, during which $[Co(N)_4(H_2O)_2]^{3+}$ was formed *in situ*. The pH of the solution was adjusted by a small amount of hydrochloric acid. The hydrolysis of ApA was initiated by adding a stock solution of ApA to the mixture. The initial concentration of ApA was $1–4 \times 10^{-4}$ mol dm^{-3}. No buffer agent was used [except for the case of the $(en)_3$ complex], since hydration water molecules on the Co^{III} complexes were sufficiently potent for the purpose. The reactions with the $(en)_3$ complex were achieved in Hepes buffers. Hydrolysis of other substrates was carried out in similar ways.

Throughout the present study, great care was taken to avoid contamination by other metal ions and natural enzymes. Highly purified water (specific resistance > 18.3 MΩ cm) was degassed and sterilized immediately before use. The samples for kinetic study were prepared under nitrogen to avoid contamination by carbon dioxide.

HPLC analysis

At an appropriate interval, an aliquot (5 μl) was analyzed by reversed-phase HPLC. Prior to HPLC injection, the specimen was pretreated as follows. First, the Co^{III} ions were reduced to the divalent state by an aqueous 0.2 mol dm^{-3} solution of Eu^{II} ion (45 μl). Then, the mixture was centrifuged to remove precipitates, and finally was treated with a pretreatment disk (Tosoh W-3-2). The Eu^{II} solution was prepared by reducing Eu_2O_3 in 1 M HCl with zinc metal, as described in the literature.[36]

The reversed-phase HPLC analysis was achieved by using a Merck LiChrospher 18(e) column. The eluent was a pH 5 buffer containing 8 vol% acetonitrile. Assignment of all the signals was confirmed by coinjection with authentic samples. Hydrolysis products (Ado, A2′p, A3′p and A>p), as well as ApA, were clearly resolved by the system. Adenine and adenylyl(2′-5′)adenosine could be also clearly detected if any, although they were not formed in the present reactions. For the analysis of A>p hydrolysis, the acetonitrile concentration in the HPLC eluent was decreased to 4 vol%. The Co^{III} complex-induced hydrolysis of A>p and other 2′,3′-cyclic monophosphates was followed for 4–5 half-lives, whereas the diribonucleotide hydrolysis was done for 1.5–2.5 half-lives [only the slowest hydrolysis by the $(en)_2$ complex was achieved for one half-life]. The reactions showed reasonable pseudo-first-order kinetics. The rate constants reported here are the averages of the results of at least duplicate runs which coincided within 5%.

Potentiometric titration

Acid dissociation constants of $[Co(trien)(OH_2)_2]^{3+}$ were determined by potentiometric titration with aqueous NaOH solution. For D_2O experiments, the reading of pH meter was corrected by the equation: pD = pH meter reading + 0.4.[37]

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26 The equilibrium constant for the complex formation between ApA and $[Co(trien)(OH_2)_2]^{3+}$ at pH 7 and 50 °C has been determined to be 0.1 mol^{-1} dm^3 by ^{31}P NMR. Thus the rate of ApA hydrolysis linearly increases with $[Co^{III}\ complex]_0$ under the conditions employed.
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28 Experimental error in these parameters is estimated to be around 10%. The smaller value of k_3 than k_4 is ascribed to electrostatic repulsion between the metal-bound hydroxide in $[Co(trien)(OH_2)(OH)]^{2+}$ and hydroxide ion. The equilibrium constants (K) for the formation of the substrate–Co^{III} complex are not taken into consideration in the calculation, since they do not affect the relative ratios of the parameters.
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