Structure–reactivity relationship for the cobalt(III) complex-catalysed hydrolysis of adenosine 3′,5′-cyclic monophosphate[1]

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Hydrolysis of adenosine 3′,5′-cyclic monophosphate (cAMP) by cobalt(III) complexes $[Co(N_4)(H_2O)_2]^{3+}$ (N_4: two diamines or one tetraamine) has been systematically studied at pH 7 and 50 °C. Both the catalytic activity and the product distribution are highly dependent on the nature of the amine ligand. The relative catalytic activities are cyclen (4000) > trien (500) > $(tme)_2$ (57) > tren (37) > $(tn)_2$ (22) > 2,3,2-tet (7) > $(en)_2$ (1) ⩾ cyclam, cth, dien. The pseudo-first-order rate constant for the cyclen complex (0.05 M) is 1.2 h^{-1} (half-life 35 min), corresponding to a 10^{10}-fold acceleration with respect to the uncatalysed reaction. Of the two P–O linkages in cAMP, the cyclen, the trien and the 2,3,2-tet complexes preferentially cleave the P–O(5′) linkage, whereas the $(tme)_2$ and the $(tn)_2$ complexes promote P–O(3′) scission. Adenosine is the main product for hydrolysis by the $(tme)_2$ complex, whereas adenosine monophosphates as the hydrolysis intermediates are accumulated in the catalysis by the trien complex.

Introduction

Adenosine 3′,5′-cyclic monophosphate (cAMP), a second messenger for cell-to-cell communication, is formed from adenosine triphosphate by adenylate cyclase, when cells are activated by external stimuli.[2] The cAMP modulates the activities of intracellular enzymes, resulting in the regulation of the bioreactions. The response terminates when the cAMP is hydrolysed by phosphodiesterase. Thus, efficient catalysts for cAMP cleavage are important in the design of artificial cell-regulating systems as well as for detailed investigation of the mechanism of information-transfer in cells.

Previously,[3] Chin and Zou reported that cAMP hydrolysis is greatly (10^8-fold) accelerated by $[Co(trien)(H_2O)_2]^{3+}$. Furthermore, Sargeson *et al.*[4] showed that $[Co(tn)_2(H_2O)_2]^{3+}$ preferentially cleaves the P–O(3′) linkage over the P–O(5′) linkage (the abbreviations of the ligands are presented in Fig. 1). These findings indicated that Co^{III} complexes have potential in artificial regulation of cell functions.[5,6] Undoubtedly, detailed information on the relationship between the structure of the complexes and their catalytic properties is crucially important.

We report here the results of a systematic study on cAMP hydrolysis by diaquatetraazacobalt(III) complexes $[Co(N_4)(H_2O)_2]^{3+}$ (N_4: two diamines or one tetraamine). Dependencies of the catalytic activity and product distribution on the structure of amine ligand (shown in Fig. 1) are quantitatively analysed. Factors governing the reactivity and the regioselectivity are clarified. Furthermore, a reaction mechanism is proposed in terms of the kinetic and spectroscopic evidence.

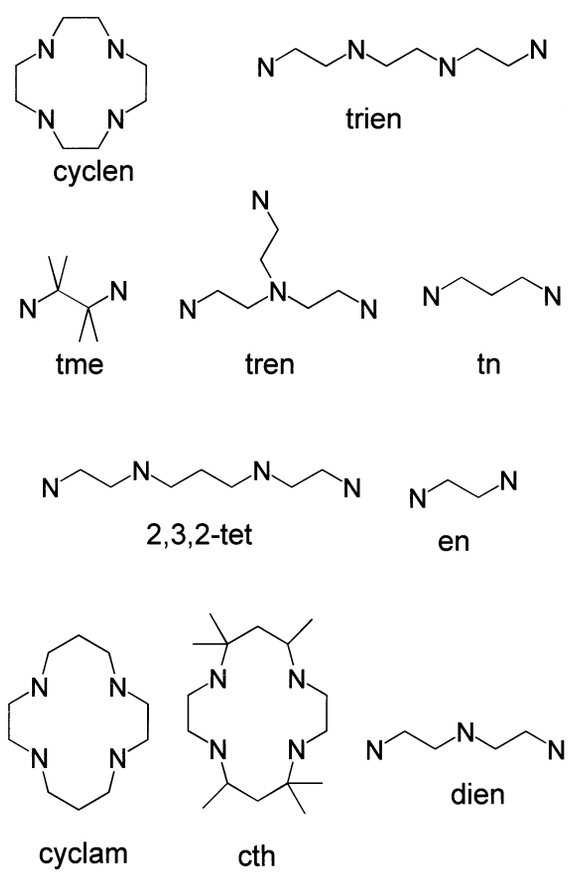

Fig. 1 Ligands used in the present study and their abbreviations. Abbreviations: cyclen, 1,4,7,10-tetraazacyclododecane; trien, triethylenetetramine; tme, 1,1,2,2-tetramethylethylenediamine; tren, tris(2-aminoethyl)amine; tn: 1,3-diaminopropane; 2,3,2-tet, *N*,*N*′-bis(2-aminoethyl)-1,3-diaminopropane; en, ethylenediamine; cyclam, 1,4,8,11-tetraazacyclotetradecane; cth, 7(*R*),14(*R*)-5,5,7,12,12,14-hexamethyl-1,4,8,11-tetraazacyclotetradecane; dien, diethylenetriamine.

Experimental

Materials

1,1,2,2-Tetramethylethylenediamine (tme)[7] and 7(*R*),14(*R*)-5,5,7,12,12,14-hexamethyl-1,4,8,11-tetraazacyclotetradecane (cth)[8] were synthesized as described previously. Other amine ligands were purchased from Nacalai or Tokyo Kasei Organic Chemicals. The Co^{III} complexes $[Co(N_4)Cl_2]^+$ were prepared according to the literature.[4,5,9–11] Aqueous solutions of Eu^{II} for the pretreatment of HPLC specimens were prepared by treating acidic solutions of Eu_2O_3 with zinc metal. Highly purified water (specific resistance > 18.3 MΩ) was sterilized immediately before use. Throughout the present study, great care was taken to avoid contamination by other metal ions and natural enzymes.

Table 1 First-order rate constants for the hydrolysis of cAMP by $[Co(N_4)(H_2O)_2]^{3+}$ at pH 7 and 50 °C as well as the 3′A:5′A ratios in the products [a]

Ligand	Rate constant/(h^{-1})	3′A:5′A ratio [b]
cyclen	1.2	5.6
trien	1.5×10^{-1}	4.5
$(tme)_2$	1.7×10^{-2}	0.40
tren	1.1×10^{-2}	1.4
$(tn)_2$	6.6×10^{-3}	0.13
2,3,2-tet	2.6×10^{-3}	5.0
$(en)_2$	3.0×10^{-4}	—
cyclam	—[c]	—
cth	—[c]	—
dien	—[c]	—
None	1.2×10^{-10} [d]	—

[a] $[Co^{III}\ complex]_0 = 5 \times 10^{-2}$ and $[cAMP]_0 = 4 \times 10^{-4}$ M. [b] The ratios of the monophosphates accumulated in the reaction mixtures. [c] Too small to be precisely determined. [d] The value from ref. 3.

Hydrolysis of cAMP

Typical procedures are as follows.[5g] To an aqueous solution of $[Co(N_4)Cl_2]^+$, 1.5 equiv. of NaOH were added. The mixture was incubated for 20 min, during which the corresponding $[Co(N_4)(H_2O)_2]^{3+}$ (existing mostly as $[Co(N_4)(OH)_2]^+$) was formed *in situ*. The pH of the solution was adjusted to a desired value by use of a small amount of hydrochloric acid. Hydrolysis of cAMP (from Sigma) was achieved at 50 °C without any additional buffer agents. The coordinated water molecules of the Co^{III} complexes (pK_a values are *ca.* 6–8) satisfactorily kept the pH constant during the reactions (the change was less than 0.1 pH unit).

HPLC analysis of cAMP hydrolysis

At an appropriate interval, an aliquot (5 μl) of the reaction solution was analysed by reversed-phase HPLC [Merck LiChrospher 18(e) column, 25 cm; eluent, pH 4 buffer containing acetonitrile (2 vol%), KH_2PO_4 (50 mM), and choline chloride (20 mM) as an ion-pair agent; detection at 258 nm]. Prior to injection to the system, the specimens were pretreated as follows.[10] First, the Co^{III} ions therein were reduced to the Co^{II} by an aqueous 0.2 M solution of Eu^{II} ion (45 μl). Then a saturated aqueous solution of KH_2PO_4 (50 μl) was added. The resultant mixture was centrifuged to remove the precipitates, and finally was treated with a pretreatment disk (Tosoh, W-3-2).

All the hydrolysis products, adenosine 3′-monophosphate (3′A), adenosine 5′-monophosphate (5′A), and adenosine (A), as well as cAMP, were clearly resolved by HPLC (the retention times were 10.7, 8.2, 33.1 and 37.1 min, respectively). The signals were assigned by coinjection with the corresponding authentic samples. All the cAMP hydrolysis fairly showed pseudo-first-order kinetics.

Spectroscopy

^{31}P NMR spectra were measured at pD 7 in D_2O on a JEOL EX-270 spectrometer with 85% H_3PO_4 as an external standard. The specimens were prepared as described above for the cAMP hydrolysis experiments, and were immediately subjected to spectroscopic measurements.

Results and discussion

Catalytic activities of Co^{III} complexes for cAMP hydrolysis

The catalytic activities of the Co^{III} complexes for the hydrolysis of cAMP at pH 7 and 50 °C are listed in Table 1. The pseudo-first-order rate constant for $[Co(cyclen)(H_2O)_2]^{3+}$ (0.05 M) is 1.2 h^{-1}, corresponding to a half-life of 35 min. Thus, a 10^{10}-fold acceleration (with respect to the uncatalysed reaction) has been accomplished (the half-life in the absence of the catalyst is estimated to be 660 000 years).[3] This is, to our knowledge, the most active Co^{III} complex for cAMP hydrolysis. The products are only 3′A, 5′A and A, confirming the hydrolytic character of the scission.

Table 2 First-order rate constants (in 10^{-2} h^{-1}) for the hydrolysis of adenosine monophosphates by $[Co(trien)(H_2O)_2]^{3+}$ and $[Co(tme)_2(H_2O)_2]^{3+}$ at pH 7 and 50 °C [a]

Ligand	3′A	5′A	cAMP
trien	4.2	1.1	15
$(tme)_2$	19	24	1.7

[a] $[Co^{III}\ complex] = 5 \times 10^{-2}$ and $[cAMP]_0 = 4 \times 10^{-4}$ M.

The hydrolysis rate depends strongly on the structure of amine ligand: cyclen > trien > $(tme)_2$ > tren > $(tn)_2$ > 2,3,2-tet > $(en)_2$. The hydrolysis rate by the cyclen complex is 4000-fold greater than that by the $(en)_2$ complex [note that even the $(en)_2$ complex accelerates the hydrolysis by more than 10^6-fold]. In contrast, the hydrolysis by the Co^{III} complexes of cyclam and cth is too slow to allow the precise determination of the rate constants. A triamine complex $[Co(dien)(H_2O)_3]^{3+}$ also has poor activity.

Preference of the P–O(3′) scission *vs.* the P–O(5′) scission

The ratio of 3′A to 5′A in the product is also notably dependent on the kind of amine ligand, as shown in Table 1. For the cyclen, the trien, the 2,3,2-tet, and the tren complexes, 3′A is the major product, showing that the P–O(5′) bond is preferentially cleaved over the P–O(3′) bond. With the $(tme)_2$ and the $(tn)_2$ complexes, however, the selectivity is reversed and 5′A is formed predominantly.† The 3′A:5′A ratios for the trien and the $(tn)_2$ complexes are in reasonable accord with the values reported by Sargeson *et al.*‡[4]

Complete hydrolysis or partial hydrolysis.

Furthermore, the amine ligand strongly affects the relative rates of the first step (cAMP ⟶ 3′A,5′A) and of the second step (3′A,5′A ⟶ A) for the cAMP hydrolysis. Thus, the distributions of the products in the reaction mixtures are significantly different from each other. When $[Co(trien)(H_2O)_2]^{3+}$ was used as catalyst, 3′A and 5′A as the intermediates were accumulated in the reaction mixture. Formation of A was not observed even after 10 h, where *ca.* 80 mol% of cAMP was cleaved. The second step is catalysed less by the complex. Similar results were obtained for the cyclen and the 2,3,2-tet complexes.

With the $(tme)_2$ complex, however, cAMP was hydrolysed straightforwardly to A without considerable accumulation of 3′A and 5′A. More than 80% of the hydrolysis products was A, when the conversion of cAMP cleavage was 50 mol%. Still higher selectivity was obtained by the $(en)_2$ complex: 50 mol% conversion of cAMP cleavage gave 97% hydrolysis product A. Hydrolysis by the tren complex was less selective, and considerable amounts of 3′A, 5′A and A were present in the reaction mixtures.

Table 2 lists the rate constants for the hydrolysis of 3′A and 5′A to A by the trien and the $(tme)_2$ complexes, which were determined by use of authentic samples of the adenosine monophosphates. For the hydrolysis of both 3′A and 5′A, the $(tme)_2$ complex has greater catalytic activity than the trien complex. In the cleavage of cAMP to 3′A and 5′A, however, the trien complex is more active than the $(tme)_2$ complex, see also Table 2. These results are consistent with the accumulation of adenosine monophosphates only with the trien complex. Subtle changes in the structure of the amine ligand give rise to a dras-

† The rate constants for the hydrolysis of 3′A and 5′A to A by the tme complex are comparable with each other (see Table 2), and thus the 3′A:5′A ratio presented in Table 1 satisfactorily reflects the regioselectivity of the scission of the P–O bonds.

‡ The 3′A:5′A ratio 0.40 for the $(tme)_2$ complex is different from the value (2) in ref. 4. The reason for the discrepancy is not clear.

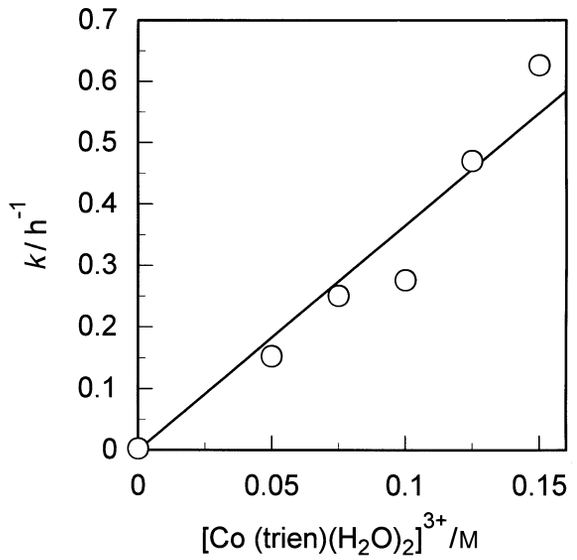

Fig. 2 Plot of the pseudo-first-order rate constant for the cAMP hydrolysis *vs.* the concentration of $[Co(trien)(H_2O)_2]^{3+}$ at pH 7 and 50 °C: $[cAMP]_0 = 4 \times 10^{-4}$ M

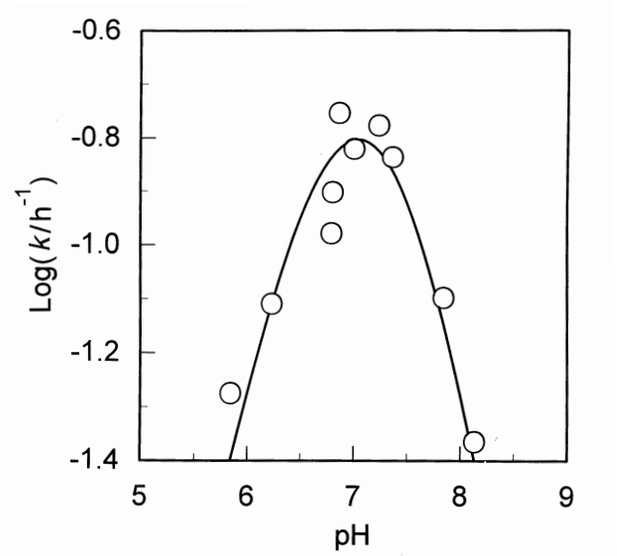

Fig. 3 The pH–rate constant profile for the $[Co(trien)(H_2O)_2]^{3+}$-catalysed hydrolysis of cAMP at 50 °C: [trien complex] = 5×10^{-2} and $[cAMP]_0 = 4 \times 10^{-4}$ M: the pK_a values of the hydration water of the Co^{III} complex are 5.9 and 8.1 [ref. 5(*e*)]

tic difference in the catalytic activities for the hydrolysis of the phospho-diesters and -monoesters.

Kinetic analysis of cAMP hydrolysis by $[Co(trien)(H_2O)_2]^{3+}$

The rate constant for the cAMP hydrolysis increases linearly with increasing concentration of $[Co(trien)(H_2O)_2]^{3+}$, as depicted in Fig. 2. Only one Co^{III} complex participates in the hydrolysis. The pH–rate constant profile is bell-shaped, giving a maximum at *ca.* pH 7 (Fig. 3). It is strongly indicated that $[Co(trien)(H_2O)(OH)]^{2+}$ is the active species [see eqn. (1): the pK_a values for the dissociation of the coordinated water molecules in the Co^{III} complex are 5.9 and 8.1].[5e]

$$[Co(trien)(H_2O)_2]^{3+} \overset{K_{a1}}{\rightleftharpoons} [Co(trien)(H_2O)(OH)]^{2+} \overset{K_{a2}}{\rightleftharpoons} [Co(trien)(OH)_2]^{1+} \quad (1)$$

Complex formation between cAMP and $[Co(trien)(H_2O)_2]^{3+}$

When $[Co(trien)(H_2O)_2]^{3+}$ was mixed with cAMP at pD 7, a new ^{31}P-signal appeared at δ 8.7. The signal of free cAMP was located at δ 2.1. The new signal is assignable to the cAMP

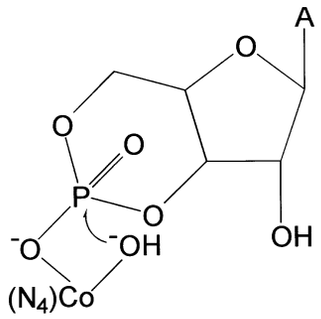

Fig. 4 The proposed mechanism for the Co^{III}-catalysed hydrolysis of cAMP

coordinating to the trien complex (monodentate coordination of a phosphate to Co^{III} complexes causes a downfield shift, in most cases, of 5–10 ppm).[12] By use of the intensities of the signals, the equilibrium constant for the complex formation between cAMP and $[Co(trien)(H_2O)_2]^{3+}$ was estimated to be *ca.* 0.1 $dm^3\ mol^{-1}$.

Reaction mechanism for the cAMP hydrolysis

The cAMP hydrolysis involves $[Co(N_4)(OH)(cAMP)]^+$ complexes, in which a hydroxide ion coordinates to the Co^{III} ion in the *cis* position to the cAMP. In these *cis* complexes, the hydroxide ion intramolecularly attacks the phosphorus atom of the cAMP (Fig. 4: a similar mechanism was proposed by Chin *et al.*).[3] Both the linear increase of the hydrolysis rate with $[Co^{III}$ complex$]_0$ (Fig. 2) and the bell-shaped pH–rate constant profile (Fig. 3) are consistent with the mechanism.§ The equilibrium constant for the complex formation between cAMP and the Co^{III} complex is so small that a saturation phenomenon is not observed under the conditions employed. The anation step is sufficiently fast and cannot be rate-limiting.[5g,h]

The argument is supported by the fact that the cyclen complex, which is rigidly fixed in a *cis* form,[13] is quite active for the reaction. In contrast, the cyclam complex, which favourably takes a *trans* form,[14] shows only marginal catalysis (see Table 1). The *cis* configuration is required for the proposed mechanism.

The order of the catalytic activities of the Co^{III} complexes for cAMP hydrolysis [cyclen > trien > tren > $(en)_2$] coincides well with that for the hydrolysis of bis(4-nitrophenyl) phosphate.[5g,h] The validity of the proposed mechanism has been further confirmed, since the latter reactions proceed *via* a similar mechanism involving an intramolecular attack by the metal-bound hydroxide toward the phosphorus atom.

Factors governing the reactivity and the regioselectivity of Co^{III} complexes

The catalytic activities of the Co^{III} complexes are primarily governed by the stability of the four-membered ring intermediate, formed on intramolecular attack by the metal-bound hydroxide towards the phosphate (Fig. 4). As proved by Chin *et al.* in the hydrolysis of bis(4-nitrophenyl)phosphate,[5h] the stability of the four-membered Co^{III} complex is highly dependent on the amine ligand. The complex becomes more stable as the bond angle opposite the four-membered ring increases.

Quite significantly and reasonably, the dependence of the reactivity on the ligand structure for the present cAMP hydrolysis [cyclen : trien : tren : $(en)_2$ = 4000 : 500 : 37 : 1] is more marked than that (170 : 18 : 3 : 1) for the hydrolysis of bis(4-

§ The pH–rate constant profile should be treated, in principle, in terms of the pK_a value of the species $[Co(N_4)(OH_2)(cAMP)]^{2+}$, in place of the value for $[Co(N_4)(OH_2)(OH)]^{2+}$. However, the present treatment is reasonably acceptable, since the kinetically determined pK_a values for the Co^{III} complex-catalysed hydrolysis of diaryl phosphates are almost identical with the corresponding values determined by potentiometric titration [ref. 5(*h*)].

nitrophenyl)phosphate. In the cAMP hydrolysis, the four-membered ring is fusing with the six-membered ring involving the phosphodiester, which is further fusing with the five-membered ring of the ribose. Thus, the stabilization of the four-membered ring, which is otherwise quite unstable, by the amine ligand should prove still more crucial for the cAMP hydrolysis to proceed efficiently.

Of the two P–O bonds in cAMP, the P–O(5′) linkage is preferentially cleaved, when the four nitrogen atoms in the ligands are covalently bound (as in cyclen, trien and 2,3,2-tet). On the other hand, $(N_2)_2$ type ligands [$(tme)_2$ and $(tn)_2$] promote the P–O(3′) scission (see Table 1). Presumably, the molecular flexibilities of the Co^{III} complexes are different from each other, which in turn affects the efficiency of pseudo-rotation in the pentacoordinated reaction intermediate.[15] In the $(tme)_2$ and the $(tn)_2$ complexes, the pseudo-rotation takes place vigorously prior to P–O scission so that the P–O(3′) scission involving a better leaving group predominates. The pK_a of the 3′-OH of ribose (*ca.* 12) is significantly smaller than that of the 5′-OH residue.[16] For the cyclen, the trien and the 2,3,2-tet complexes, however, the pseudo-rotation is less efficient due to the tetracoordinating chelation of the ligands. Under these conditions, the direction of the nucleophilic attack by the Co^{III}-bound hydroxide toward the phosphorus atom, at least partially, governs the regioselectivity.¶

There is no correlation between the acid properties of the Co^{III}-bound water molecules and the catalytic activities of the complexes; the pK_{a1} and pK_{a2} values of the Co^{III} complexes are as follows: 5.6 and 8.0 for cyclen;[5h] 5.9 and 8.1 for trien;[5e] 4.8 and 8.7 for tme;[5e] 5.5 and 8.0 for tren;[5h] 5.6 and 8.1 for tn.[5e] The possibility that the catalytic properties are determined by these acid–base properties is ruled out.

In conclusion, both the catalytic activities and the regioselectivities of Co^{III} complexes are markedly dependent on the structure of amine ligand. Steric factors are predominant. The present systematic information sheds light on the molecular design of Co^{III} complexes useful for the artificial regulation of cell functions.

¶ The attack from the opposite side of the 5′-OH residue might be sterically and/or electrostatically preferable, although the detail is not clear.

The argument presented here is one of the possible mechanisms for the regioselective scission of cAMP. Information on the life-time of the pentacoordinated intermediate and on the rate of its pseudo-rotation is required for further discussion.

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