Enormous Acceleration by Cerium(IV) for the Hydrolysis of Nucleoside 3′,5′-Cyclic Monophosphates at pH 7

Jun Sumaoka, Sachiko Miyama and Makoto Komiyama*

Department of Industrial Chemistry, Faculty of Engineering, the University of Tokyo, Hongo, Tokyo 113, Japan

At pH 7 and 30 °C, 3′,5′-cyclic monophosphates of adenosine and guanosine are promptly hydrolysed by $Ce(NH_4)_2(NO_3)_6$ (10^{-2} mol dm^{-3}), with half-lives of 7 and 16 s, respectively.

3′,5′-Cyclic monophosphates of adenosine and guanosine (cAMP and cGMP) regulate the functions of cells through modulation of the activities of the enzymes therein.[1,2] Thus their nonenzymatic hydrolysis should have various potential applications.[3,4] Previously we showed that $CeCl_3$ is highly active for the hydrolysis of cAMP.[5] However, the hydrolysis is remarkable only in alkaline solutions (pH > 8), and is rather slow at the physiological pH of 7 (the half-life is more than 100 h at pH 7 and 30 °C). Here we report that the Ce^{IV} ion hydrolyses both cAMP and cGMP much more promptly ($>10^4$ fold) than Ce^{III} under physiological conditions. Furthermore, these cyclic phosphates are also efficiently hydrolysed by homogeneous solutions composed of Ce^{IV} and γ-cyclodextrin (γ-CyD).

Remarkable catalysis by $Ce(NH_4)_2(NO_3)_6$ (0.01 mol dm^{-3}) for the hydrolysis of cAMP at pH 7 and 30 °C has been clearly evidenced by reversed-phase HPLC [Fig. 1(*a,b*)]. The pseudo-first-order rate constant is 6.1 min^{-1} (the half-life is only 7 s), corresponding to more than a 10^{13} fold acceleration (the half-life in the absence of Ce^{IV} is estimated to be 3×10^6 years).[3] The ratio of adenosine 3′-phosphate to adenosine 5′-phosphate in the product is 7.3 : 1, showing a preferential P–O(5′) scission. Absence of concurrent oxidative cleavage of the ribose has been confirmed both by HPLC and by ^{1}H NMR spectroscopy. cGMP as well as 3′,5′-cyclic monophosphates of 2′-deoxyadenosine and thymidine is also hydrolysed efficiently (Table 1).

The rate of cAMP hydrolysis by Ce^{IV} is almost independent of pH in the region pH 2–8. This contrasts strongly with the fact that the rate by $CeCl_3$ drastically decreases with decreasing pH (a decrease of pH from 8 to 7 resulted in a 10^4 fold decrease in the hydrolysis rate).[5] Thus the hydrolysis by $CeCl_3$ is only marginal at pH 7 even over 3 days [Fig. 1(*d*)].† The rate constant (1.0×10^{-4} min^{-1} when $[CeCl_3]_0 = 0.01$ mol dm^{-3}) is reduced by a factor of 6×10^4 relative to Ce^{IV}. The activities of other trivalent lanthanide ions at pH 7 are similar to that of Ce^{III}, whilst the activity of Mg^{2+}, Zn^{2+}, Al^{3+} or Fe^{3+} is virtually nil.

In the reaction mixtures with $Ce(NH_4)_2(NO_3)_6$ some precipitation occurs, probably of the metal hydroxide. However, totally homogeneous solutions can be prepared by the addition of γ-CyD, as recently found by us (see following paper).[6] The resultant homogeneous solutions are sufficiently active for cyclic phosphate hydrolysis [see Fig. 1(*c*) and Table 1), and the rate is independent of pH from pH 2–8.

The hydrolysis rate is proportional to $[Ce^{IV}]_0$ either in the presence of γ-CyD or in its absence. Presumably the hydrolysis proceeds *via* an intramolecular attack by the hydroxide ion coordinated to Ce^{IV} toward the phosphate bound to the same

Table 1 Rate constants (k/min^{-1}) for the hydrolysis of 3′,5′-cyclic monophosphates of nucleosides by $Ce(NH_4)_2(NO_3)_6$ (0.01 mol dm^{-3}) at pH 7 and 30 °C

Nucleoside	Without γ-CyD	With γ-CyD (0.05 mol dm^{-3})
Adenosine	6.1	0.16
Guanosine	2.6	0.12
2′-Deoxyadenosine	3.9	0.081
Thymidine	1.2	0.11

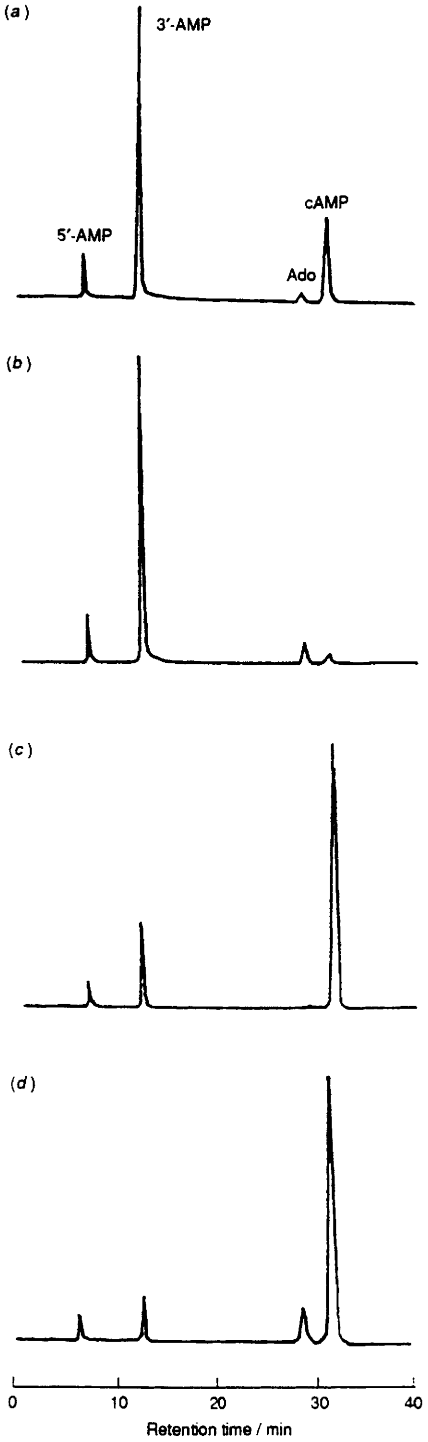

Fig. 1 Reversed-phase HPLC patterns for the hydrolysis of cAMP at pH 7 (Hepes buffer) and 30 °C: (*a*) by $Ce(NH_4)_2(NO_3)_6$ (0.01 mol dm^{-3}) after 15 s; (*b*) after 30 s; (*c*) by combination of $Ce(NH_4)_2(NO_3)_6$ (0.01 mol dm^{-3}) and γ-CyD (0.05 mol dm^{-3}) after 1 min; (*d*) by $CeCl_3$ (0.01 mol dm^{-3}) after 3 days

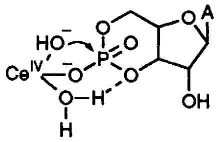

Fig. 2 Proposed mechanism for the cAMP hydrolysis

centre,[7] as depicted in Fig. 2. Another coordinated water bound to the Ce^{IV} can cooperatively function as an acid catalyst. The significant catalysis of Ce^{IV} is probably associated with the small pK_a (*ca.* 0) of coordinated water (the corresponding value of Ce^{III} and other lanthanide(III) ions are 8–9 and thus the concentration of the metal-bound hydroxide ion rapidly decreases with decreasing pH).[8,9] Furthermore, the tetravalent positive charge stabilizes the negatively charged transition state for the phosphodiester hydrolysis.

When molecular oxygen was removed from the mixture by repeated freeze–thaw cycles, the rate of hydrolysis was unchanged. Addition of hydrogen peroxide (0.01 mol dm^{-3}) retarded the hydrolysis 10-fold. The possibility of participation of either molecular oxygen or hydrogen peroxide (even if formed *in situ*) in the present hydrolysis is ruled out.‡

In conclusion, biologically important cAMP and cGMP have been hydrolysed efficiently at pH 7 nonenzymatically for the first time, by use of Ce^{IV}. Hydrolysis can be achieved in totally homogeneous solutions by the addition of γ-CyD. A potential use of Ce^{IV} for artificial regulation of cell functions can be envisaged.

This work was partially supported by a Grant-in-Aid for Scientific Research on Priority Areas from the Ministry of Education, Science and Culture.

Received, 21st March 1994; Com. 4/01651C

Footnotes

† No oxidation of Ce^{III} to Ce^{IV} occurred in the reaction mixture as shown by titration.

‡ Recently it was proposed that, in cerium(III) salt-induced DNA hydrolysis, hydrogen peroxide and Ce^{IV} are formed from Ce^{III} and molecular oxygen and that DNA hydrolysis proceeds as *via* a cerium(IV)-promoted nucleophilic attack by the hydrogen peroxide; see, K. B. Takasaki and J. Chin, *J. Am. Chem. Soc.*, 1994; **116**, 1121. The mechanism however is not applicable in the present cAMP hydrolysis.

References

1 H. Dugas and C. Penney, *Bioorganic Chemistry*, Springer-Verlag, New York, 1981 and references therein.
2 M. Waterman, G. H. Murdoch, R. M. Evans and M. G. Rosenfeld, *Science*, 1985, **229**, 267.
3 J. Chin and X. Zou, *Can. J. Chem.*, 1987, **65**, 1882.
4 K. Yonezawa, Y. Matsumoto and M. Komiyama, *Chem. Express* 1991, **6**, 965.
5 J. Sumaoka, M. Yashiro and M. Komiyama, *J. Chem. Soc., Chem. Commun.*, 1992, 1707.
6 M. Yashiro, S. Miyama, T. Takarada and M. Komiyama, following paper.
7 The hydrolysis of activated phosphate esters by trivalent lanthanide ions and their complexes proceeds by similar intracomplex reactions: R. W. Hay and N. Govan, *J. Chem. Soc., Chem. Commun.*, 1990, 714; M. Komiyama, K. Matsumura and Y. Matsumoto, *J. Chem. Soc., Chem. Commun.*, 1992, 640; J. R. Morrow, L. A. Buttrey, V. M. Shelton and K. A. Berback, *J. Am. Chem. Soc.*, 1992, **114**, 1903 and references therein.
8 J. Burgess, *Metal Ions in Solution*, Ellis Horwood, Chichester 1978, p. 267.
9 R. B. Martin, *Met. Ions Biol. Syst.*, 1984, **17**, 1.