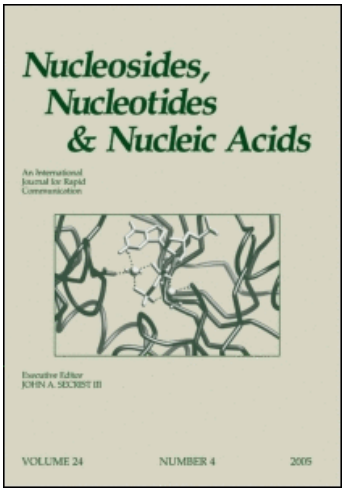


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Active Species for Ce(IV)-Induced Hydrolysis of Phosphodiester Linkage in cAMP AND DNA

Jun Sumaoka[a]; Kenichiro Furuki[a]; Yuki Kojima[a]; Masahiko Shibata[b]; Kimihiko Hirao[b]; Naoya Takeda[c]; Makoto Komiyama[a]
[a] Research Center for Advanced Science and Technology, The University of Tokyo, Komaba, Tokyo, Japan [b] Department of Applied Chemistry, Graduate School of Engineering, The University of Tokyo, Hongo, Tokyo, Japan [c] Department of Chemistry and Biotechnology, Graduate School of Engineering, The University of Tokyo, Hongo, Tokyo, Japan

To cite this Article Sumaoka, Jun , Furuki, Kenichiro , Kojima, Yuki , Shibata, Masahiko , Hirao, Kimihiko , Takeda, Naoya and Komiyama, Makoto(2006) 'Active Species for Ce(IV)-Induced Hydrolysis of Phosphodiester Linkage in cAMP AND DNA', Nucleosides, Nucleotides and Nucleic Acids, 25: 4, 523 — 538
To link to this Article: DOI: 10.1080/15257770600684209
URL: http://dx.doi.org/10.1080/15257770600684209

Nucleosides, Nucleotides, and Nucleic Acids, 25:523–538, 2006

ISSN: 1525-7770 print / 1532-2335 online
DOI: 10.1080/15257770600684209

Taylor & Francis
Taylor & Francis Group

ACTIVE SPECIES FOR Ce(IV)-INDUCED HYDROLYSIS OF PHOSPHODIESTER LINKAGE IN cAMP AND DNA

Jun Sumaoka, Kenichiro Furuki, and Yuki Kojima □ *Research Center for Advanced Science and Technology, The University of Tokyo, Komaba, Tokyo, Japan*

Masahiko Shibata and Kimihiko Hirao □ *Department of Applied Chemistry, Graduate School of Engineering, The University of Tokyo, Hongo, Tokyo, Japan*

Naoya Takeda □ *Department of Chemistry and Biotechnology, Graduate School of Engineering, The University of Tokyo, Hongo, Tokyo, Japan*

Makoto Komiyama □ *Research Center for Advanced Science and Technology, The University of Tokyo, Komaba, Tokyo, Japan*

□ *The hydrolysis of cyclic adenosine 3′,5′-monophosphate and 2′-deoxythymidylyl(3′-5′)2′-deoxythymidine by $Ce(NH_4)_2(NO_3)_6$ was kinetically studied. The rate of hydrolysis was fairly proportional to the concentration of $[Ce_2^{IV}(OH)_4]^{4+}$, showing that this is the catalytically active species. According to quantum-chemical calculation, the two Ce(IV) ions in this $[Ce_2^{IV}(OH)_4]^{4+}$ cluster are bridged by two OH residues. Upon the complex formation with $H_2PO_4^-$ (a model compound for the phosphodiesters), these two Ce(IV) ions bind the two oxygen atoms of the substrate and enhance the electrophilicity of the phosphorus atom. The catalytic mechanism of Ce(IV)-induced hydrolysis of phosphodiesters has been proposed on the basis these results.*

Keywords Artificial nuclease; Cerium; DNA cleavage; Enzyme models; Phosphodiester hydrolysis

Received 29 December 2005; accepted 9 February 2006.

This work was partially supported by the Bio-oriented Technology Research Advancement Institution. The support by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan is also acknowledged.

This article is dedicated to Professor Eiko Ohtsuka on the occasion of her 70th birthday.

Current address for Naoya Takeda: Consolidated Research Institute for Advanced Science and Medical Care, Waseda University, Shinjuku-ku, Tokyo 162-0041, Japan.

Address correspondence to Makoto Komiyama, Research Center for Advanced Science and Technology, The University of Tokyo, Komaba, Tokyo 153-8904, Japan. E-mail: komiyama@mkomi.rcast.u-tokyo.ac.jp

INTRODUCTION

Non-enzymatic hydrolysis of biologically important phosphodiesters such as cyclic adenosine 3′,5′-monophosphate (cAMP), DNA, RNA, and phospholipids has been attracting increasing interest, because of potential applications to molecular biology, biotechnology, therapy, and others.[1] Superb catalysts for the hydrolysis of RNA have been successfully obtained.[2] However, other phosphoesters, especially cAMP and DNA, are so stable that few catalysts can hydrolyze them at reasonable rates under physiological conditions (the half-lives of the phosphodiester linkages of cAMP and DNA in the absence of catalysts are estimated to be 400 thousand[3] and 200 million years,[4] respectively). About 10 years ago, it was found that Ce(IV) ion is remarkably active for the hydrolysis of them under physiological conditions.[5–10] No concurrent oxidation of cAMP and DNA was detectable, indicating a strong potential for practical applications. The acceleration accomplished by this metal ion was as large as 10^{11}–10^{13} fold. Quite interestingly and importantly, the catalytic activity of Ce(IV) for the hydrolysis of both cAMP and DNA is overwhelmingly greater than those of other metal ions. Furthermore, both reactions proceed via nucleophillic attack by external OH^- (or its relevant species), which is in contrast with intramolecular attack by 2′-OH in RNA hydrolysis (transesterification).[5,7] Thus, they have many features in common. Several spectroscopic studies have been already made on Ce(IV)/phosphodiester complexes.[11,12] Nevertheless, the species responsible for the catalysis has not yet been pinned down and the mechanism of catalysis by Ce(IV) is not completely clear. One of the most significant obstacles is the fact that the Ce(IV) ions form complicated gel of metal hydroxide at physiological pH, which has been preventing detailed analysis of the reactions.

However, we have now found that highly acidic solutions of Ce(IV) are free from the precipitation of gel and are appropriate for kinetic analysis. Significantly, these solutions are sufficiently active for the hydrolysis of cAMP and DNA, and their catalytic activities are similar to those of the metal hydroxide gel obtained at neutral pH. Furthermore, the concentrations of all the Ce(IV)-derived species in these solutions can be calculated by using the equilibrium constants which are available in the literature.[13] In this article, the hydrolysis of cAMP and 2′-deoxythymidylyl(3′-5′)2′-deoxythymidine (TpT) in these acidic solutions is kinetically studied in detail. The dependence of hydrolysis rate on the concentration of each of the Ce(IV)-derived species is analyzed, and the catalytically active species is determined. The geometric structure and electric properties of this active species, as well as those of its complex with a phosphodiester, are investigated by *ab initio* quantum-chemical calculation. The reaction mechanism for the Ce(IV)-induced hydrolysis of phosphodiesters is proposed in terms of these results.

RESULTS AND DISCUSSION

Hydrolysis of cAMP and DNA by $Ce(NH_4)_2(NO_3)_6$ in Acidic Solutions

When the pH of $Ce(NH_4)_2(NO_3)_6$ solution was lower than 2.5, there existed no precipitates of Ce(IV) hydroxide in the solution. The absence of colloidal particles in these solutions was confirmed by light-scattering photometry using the instrument which can detect the particles, if any, of diameter 15 Å or larger. The pseudo-first-order rate constant for the disappearance of cAMP in the presence of $Ce(NH_4)_2(NO_3)_6$ (1 *mM*) at pH 2.0 and 30°C is 1.5×10^{-3} s^{-1}.[14] This value is smaller than the value (4.7×10^{-3} s^{-1}) at pH 7.0, but the difference is only 3-fold. The major product was adenosine 3′-monophosphate, exactly as was the case at pH 7.0.[15] The 3′-monophosphate, as well as the 5′-monophosphate, was gradually hydrolyzed to adenosine as the final product. No oxidative cleavage of the nucleobase and the ribose was detectable. According to the redox titration described in Experimental section, all the Ce(IV) ions in the solutions retained their tetravalent states throughout the cAMP hydrolysis. No redox reactions took place in the mixtures. Cyclic 3′,5′-monophosphates of guanosine, cytidine, and uridine were also promptly hydrolyzed by $Ce(NH_4)_2(NO_3)_6$ at pH 2.0.

The pH-rate constant profile for the hydrolysis of the dinucleotide TpT by $Ce(NH_4)_2(NO_3)_6$ (10 *mM*) was also flat. The rate constant at pH 2.0 and 50°C was 1.1×10^{-5} s^{-1}, whereas the value at pH 7.0 was 5.3×10^{-5} s^{-1}. The product was mostly thymidine (pT and Tp, formed by the hydrolysis of TpT, were rapidly hydrolyzed and not much accumulated). Thymine and other oxidation products were not formed under these conditions (only when the pH was lower than 1.4, thymine was slightly formed due to the oxidation and thus the present kinetic studies were carried out at pH > 1.4). Essential features of the hydrolyses of cAMP and TpT by $Ce(NH_4)_2(NO_3)_6$ in acidic solutions are similar to those at pH 7.[5,7] In the absence of $Ce(NH_4)_2(NO_3)_6$, neither cAMP nor TpT was hydrolyzed to a detectable extent, as expected from their stabilities described above.

Kinetic Analysis of the Hydrolysis of cAMP by $Ce(NH_4)_2(NO_3)_6$

The equilibrium constants for the solvolysis of Ce(IV) were previously reported at 25°C and $[NaClO_4] = 3.0$ *M* (see Eqs. (1–7)).[13] Thus the concentrations of the Ce(IV)-derived species formed in reaction mixtures ($[Ce^{IV}]^{4+}$, $[Ce^{IV}(OH)]^{3+}$, $[Ce^{IV}(OH)_2]^{2+}$, $[Ce_2^{IV}(OH)_2]^{6+}$, $[Ce_2^{IV}(OH)_3]^{5+}$, $[Ce_2^{IV}(OH)_4]^{4+}$, and $[Ce_6^{IV}(OH)_{12}]^{12+}$) can be calculated by using $Q_{1,1}$, $Q_{1,2}$, $Q_{2,2}$, $Q_{2,3}$, $Q_{2,4}$, and $Q_{6,12}$ values. Here the $Q_{x,y}$ is defined by Eq. (1).

$$x\,Ce^{IV} + y\,H_2O \rightleftharpoons \left[Ce_x^{IV}(OH)_y\right]^{(4x-y)+} + yH^+$$

$$Q_{x,y} = \frac{\left[\left[Ce_x^{IV}(OH)_y\right]^{(4x-y)+}\right][H^+]^y}{[Ce^{IV}]^x} \quad (1)$$

The present kinetic analysis was achieved under the same conditions where these $Q_{x,y}$ values were determined.

$$Ce^{IV} + H_2O \rightleftharpoons [Ce^{IV}(OH)]^{3+} + H^+ \qquad \log Q_{1,1} = 1.1 \quad (2)$$

$$Ce^{IV} + H_2O \rightleftharpoons \left[Ce^{IV}(OH)_2\right]^{2+} + 2H^+ \qquad \log Q_{1,2} = 0.3 \quad (3)$$

$$2Ce^{IV} + 2H_2O \rightleftharpoons \left[Ce_2^{IV}(OH)_2\right]^{6+} + 2H^+ \qquad \log Q_{2,2} = 3.6 \quad (4)$$

$$2Ce^{IV} + 3H_2O \rightleftharpoons \left[Ce_2^{IV}(OH)_3\right]^{5+} + 3H^+ \qquad \log Q_{2,3} = 4.1 \quad (5)$$

$$2Ce^{IV} + 4H_2O \rightleftharpoons \left[Ce_2^{IV}(OH)_4\right]^{4+} + 4H^+ \qquad \log Q_{2,4} = 3.5 \quad (6)$$

$$6Ce^{IV} + 12H_2O \rightleftharpoons \left[Ce_6^{IV}(OH)_{12}\right]^{12+} + 12H^+ \qquad \log Q_{6,12} = 15.4 \quad (7)$$

The equilibrium concentrations of these Ce(IV)-derived species under typical reaction conditions (pH 2.0 and $[Ce(NH_4)_2(NO_3)_6]_0 = 1$ *mM*) are presented in Table 1. The abundance of these species is in the following order: $[Ce^{IV}(OH)_2]^{2+} > [Ce_2^{IV}(OH)_4]^{4+} \gg [Ce^{IV}(OH)]^{3+} > [Ce_2^{IV}(OH)_3]^{5+} \gg [Ce_6^{IV}(OH)_{12}]^{12+} \gg [Ce_2^{IV}(OH)_2]^{6+} \approx [Ce^{IV}]^{4+}$.

The hydrolysis of cAMP in acidic solutions was sufficiently fast and fair first-order kinetics was always obtained up to high conversion. As shown by the open circles in Figure 1a, the pseudo-first-order rate constant k_{obs} monotonously increased with increase in the initial concentration of $Ce(NH_4)_2(NO_3)_6$. Here, the pH was kept constant at 2.0. All the experimental points fairly fit the solid line (vi), which shows the equilibrium concentration of the bimetallic hydroxo-cluster $[Ce_2^{IV}(OH)_4]^{4+}$. The dotted lines (iv) and (v) referring to the equilibrium concentrations of

TABLE 1 Distribution of Ce^{IV}-Derived Species at pH 2.0, 25°C, and $[Ce(NH_4)_2(NO_3)_6]_0 = 1$ *mM* ($[NaClO_4] = 3.0$ *M*)

Ce^{IV}-derived species	Mole fraction (%)
$[Ce^{IV}]^{4+}$	0.0034
$[Ce^{IV}(OH)]^{3+}$	4.2
$[Ce^{IV}(OH)_2]^{2+}$	67
$[Ce_2^{IV}(OH)_2]^{6+}$	0.0035
$[Ce_2^{IV}(OH)_3]^{5+}$	1.1
$[Ce_2^{IV}(OH)_4]^{4+}$	28
$[Ce_6^{IV}(OH)_{12}]^{12+}$	0.1

$[Ce_2^{IV}(OH)_2]^{6+}$ and $[Ce_2^{IV}(OH)_3]^{5+}$, respectively, have similar shapes as the line (vi). These three species are the candidates for the catalytically active species. On the other hand, the lines (i–iii) for $[Ce^{IV}]^{4+}$, $[Ce^{IV}(OH)]^{3+}$, and $[Ce^{IV}(OH)_2]^{2+}$ were too flat to satisfy the experimental results, and the line (vii) for $[Ce_6^{IV}(OH)_{12}]^{12+}$ was too steep. These four species cannot be the active species. Note that the shapes of these lines are concretely determined a priori only by the $Q_{x,y}$ values, pH, and $[Ce(NH_4)_2(NO_3)_6]_0$. On the other hand, the positions of lines are vertically movable by varying the catalytic rate constants of the corresponding Ce(IV)-derived species.

The pH dependence of the rate constant of hydrolysis is shown by the open circles in Figure 1b ($[Ce(NH_4)_2(NO_3)_6]_0$ is kept constant at 1 *mM*). The profile is rather flat from pH 1.4 to 2.1. All the experimental points are consistent with the shapes of the theoretical lines for the equilibrium concentrations of $[Ce^{IV}(OH)_2]^{2+}$ (iii), $[Ce_2^{IV}(OH)_4]^{4+}$ (vi), and $[Ce_6^{IV}(OH)_{12}]^{12+}$ (vii). The concentrations of the other four species rapidly decrease with increasing pH and are inconsistent with the experimental data (the line (i) and the line (iv) are completely superimposed with each other). Of the seven Ce(IV)-derived species in the mixtures, only the tetracationic bimetallic hydroxo-cluster $[Ce_2^{IV}(OH)_4]^{4+}$ agrees with both of the analyses in Figures 1a and 1b, which are independent from each other. It is concluded that this species should be responsible for the hydrolysis of cAMP by $Ce(NH_4)_2(NO_3)_6$.[16]

The assignment of $[Ce_2^{IV}(OH)_4]^{4+}$ to the catalytically active species for the hydrolysis has been further confirmed by Figure 1c. Here, both $[Ce(NH_4)_2(NO_3)_6]_0$ and pH were varied randomly and independently. As shown by the open circles, the plot of hydrolysis rate vs. the equilibrium concentration of $[Ce_2^{IV}(OH)_4]^{4+}$ fairly fits the solid straight line which passes through the origin. From its slope, the second-order catalytic rate constant of this dinuclear cluster is evaluated to be 2.7 M^{-1} s^{-1}. However, there is no linear relationship between the hydrolysis rate and the concentration of $[Ce^{IV}(OH)_2]^{2+}$ (the closed circles). Although this mononuclear Ce(IV) species is abundant in the reaction mixtures, it cannot be the active species.[16] The ternary reaction system involving two of these monomeric Ce(IV) species (and the substrate) is unfavorable due to notable loss of entropy. This argument is consistent with the following theoretical studies. No linear relationship was observed between k_{obs} and the concentration of any other Ce(IV)-derived species.

Kinetic Analysis of the Hydrolysis of TpT by $Ce(NH_4)_2(NO_3)_6$

Similar kinetic analysis was carried out on the hydrolysis of TpT by $Ce(NH_4)_2(NO_3)_6$ at 25°C and $[NaClO_4] = 3.0$ *M* (Figure 2). Only hydrolytic product (thymidine) was formed (vide ante). In Figure 2a, $[Ce(NH_4)_2(NO_3)_6]_0$ was varied with the pH kept constant, whereas Figure 2b

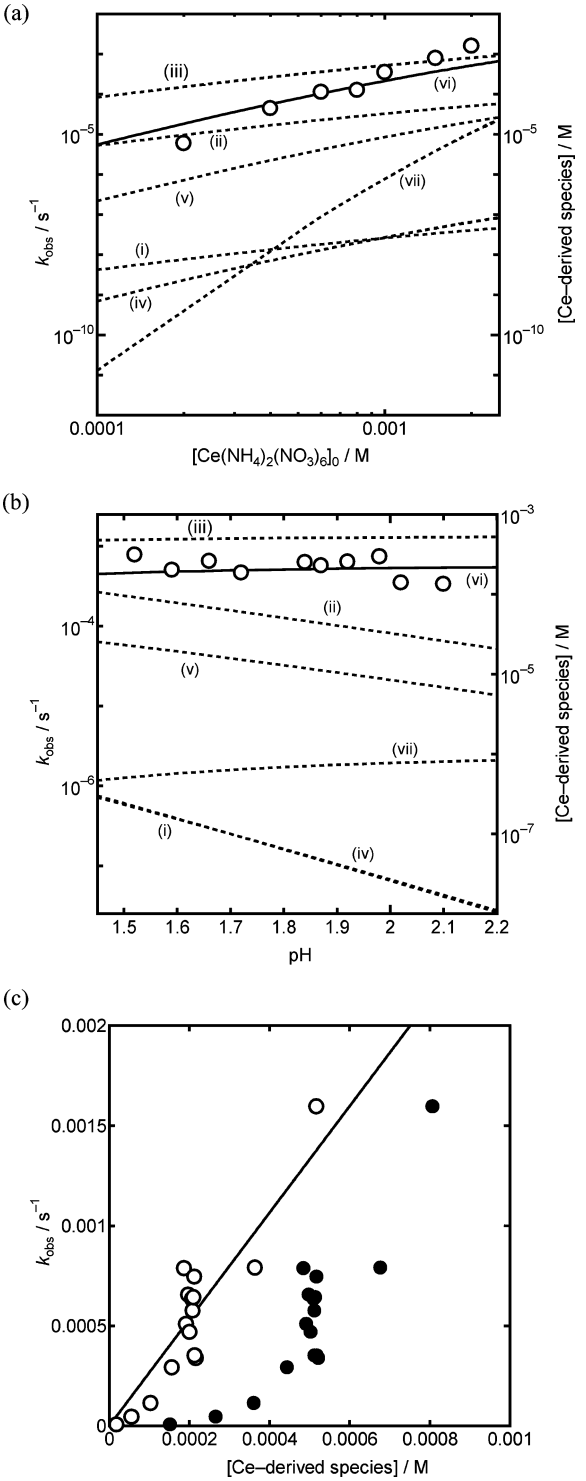
shows the pH dependence of hydrolysis rate ($[Ce(NH_4)_2(NO_3)_6]_0$ is kept constant). In both figures, the experimental points fairly fit the theoretical line (the solid one) showing the equilibrium concentration of the bimetallic hydroxo-cluster $[Ce_2^{IV}(OH)_4]^{4+}$. No other Ce(IV)-derived species satisfy the experimental results. Consistently, k_{obs} linearly increased with increasing concentration of $[Ce_2^{IV}(OH)_4]^{4+}$ when both $[Ce(NH_4)_2(NO_3)_6]_0$ and pH were varied independently (the open circles in Figure 2c). On the other hand, the plot of k_{obs} vs. $[Ce^{IV}(OH)_2]^{2+}$ never fit a straight line which passes through the origin (the closed circles). It is strongly indicated that $[Ce_2^{IV}(OH)_4]^{4+}$ is also the catalytic species for TpT hydrolysis, as is the case in the hydrolysis of cAMP.

Quantum-Chemical Studies on the Catalytically Active $[Ce_2^{IV}(OH)_4]^{4+}$ Cluster and Its Complex with Phosphodiester

According to ab initio calculation, $[Ce_2^{IV}(OH)_4]^{4+}$ is stable when the two Ce(IV) ions therein are bridged by two hydroxide groups. Its optimized structure is presented in Figure 3a.[17] All of the two Ce(IV) atoms and these two bridging OH groups (both the oxygen atoms and the hydrogen atoms) are located in one plane. The whole structure of the cluster has C2 symmetry with respect to the straight line connecting the two OH groups. The distance between the two Ce(IV) atoms is 3.848 Å. This value is fairly in accord with the Ce-Ce distance (3.6 Å) in the 2:1 Ce(IV) complex of diphenyl phosphate, which was evaluated by EXAFS study.[11] It is noteworthy that a bimetallic Ce(IV) cluster of the same composition is quite unstable when there exists only one OH bridge between the two Ce(IV) atoms and another OH is bound to either of the two Ce(IV) ions. Upon optimizing the geometry of this bimetallic cluster by the quantum-chemical calculation, the OH-bridge was spontaneously cleaved and accordingly the cluster was decomposed to two $[Ce^{IV}(OH)_2]^{2+}$ molecules.

As a model compound of phosphodiesters for the calculation, $H_2PO_4^-$ was employed since the phosphoesters mostly exist as monoanion under the reaction conditions (the pK_a value of the phosphodiester linkage of TpT is

FIGURE 1 Plots of logarithm of the pseudo-first-order rate constant of cAMP hydrolysis at 25°C ($[NaClO_4] = 3.0\ M$) vs. (a) the initial concentration of $Ce(NH_4)_2(NO_3)_6$ and (b) the pH. The pH was kept constant at 2.0 in (a), whereas $[Ce(NH_4)_2(NO_3)_6]_0$ was constant at 1 *mM* in (b). The solid line (vi) is the theoretical one showing the concentration of the proposed active species $[Ce_2^{IV}(OH)_4]^{4+}$ (the right-handed ordinate). The dotted lines are the theoretical ones for (i) $[Ce]^{4+}$; (ii) $[Ce^{IV}(OH)]^{3+}$; (iii) $[Ce^{IV}(OH)_2]^{2+}$; (iv) $[Ce_2^{IV}(OH)_2]^{6+}$; (v) $[Ce_2^{IV}(OH)_3]^{5+}$, and (vii) $[Ce_6^{IV}(OH)_{12}]^{12+}$. All the theoretical lines were calculated using the equilibrium constants that were determined under the same conditions as the reaction conditions (25°C and $[NaClO_4] = 3.0\ M$).[13] In (c), both $[Ce(NH_4)_2(NO_3)_6]_0$ and pH were varied independently from each other, and the rate constants were plotted vs. the equilibrium concentrations of $[Ce_2^{IV}(OH)_4]^{4+}$ (the open circles) and $[Ce^{IV}(OH)_2]^{2+}$ (the closed circles).



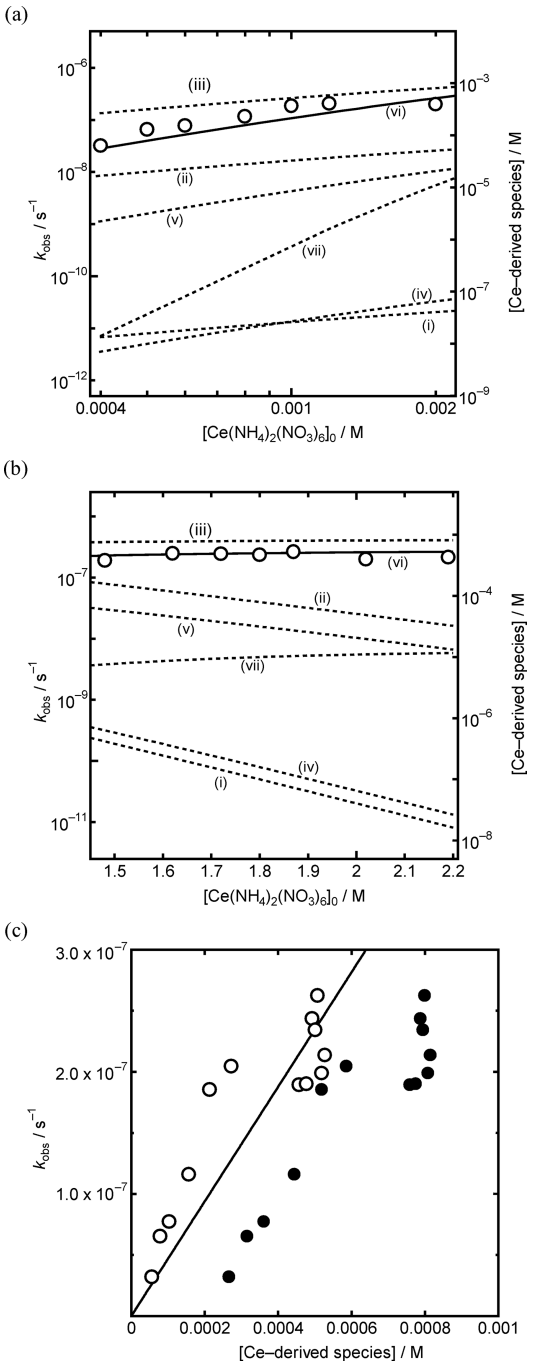


FIGURE 2 Plots of logarithm of the pseudo-first-order rate constant of TpT hydrolysis at 25°C ($[NaClO_4] = 3.0\ M$) vs. (a) the initial concentration of $Ce(NH_4)_2(NO_3)_6$ and (b) the pH. The pH was kept constant at 2.0 in (a), whereas $[Ce(NH_4)_2(NO_3)_6]_0$ was constant at 10 mM in (b). The solid and the dotted lines are the theoretical lines (see the legend for Figure 1). In (c), the rate constants were plotted vs. the equilibrium concentrations of $[Ce_2^{IV}(OH)_4]^{4+}$ (the open circles) and $[Ce^{IV}(OH)_2]^{2+}$ (the closed circles) using the data in (a) and (b).

0.4, according to the previous titration by ^{31}P-NMR).[5a] Figure 3b shows the optimized structure of the complex between $H_2PO_4^-$ and the $[Ce_2^{IV}(OH)_4]^{4+}$ cluster having two bridging OH groups (the structure depicted in Figure 3a). This complex is stable, and the free energy change for its formation, evaluated by the theoretical calculation, is -20.31 kcal mol^{-1}. The two oxygen atoms of $H_2PO_4^-$ are coordinated to each of the two Ce(IV) atoms, and the Ce-O distance for both of the coordination is 2.132 Å. The charge (+2.57) on the phosphorus atom in this complex is remarkably greater than the value of free $H_2PO_4^-$ (+1.89), confirming that the electrons on this atom are notably withdrawn by the Ce(IV) ions. As the result, the electrophilicity of this reaction center is enhanced. The positive charges of two Ce atoms in the complex are 2.26 and 2.27. Upon the complex formation, the symmetric structure of the bimetallic Ce(IV) cluster in Figure 3a is slightly distorted so that the two Ce(IV) atoms are pushed towards the $H_2PO_4^-$ and form two coordination bonds with their two oxygen atoms. However, the Ce-Ce distance (3.727 Å) is almost unchanged during these processes.

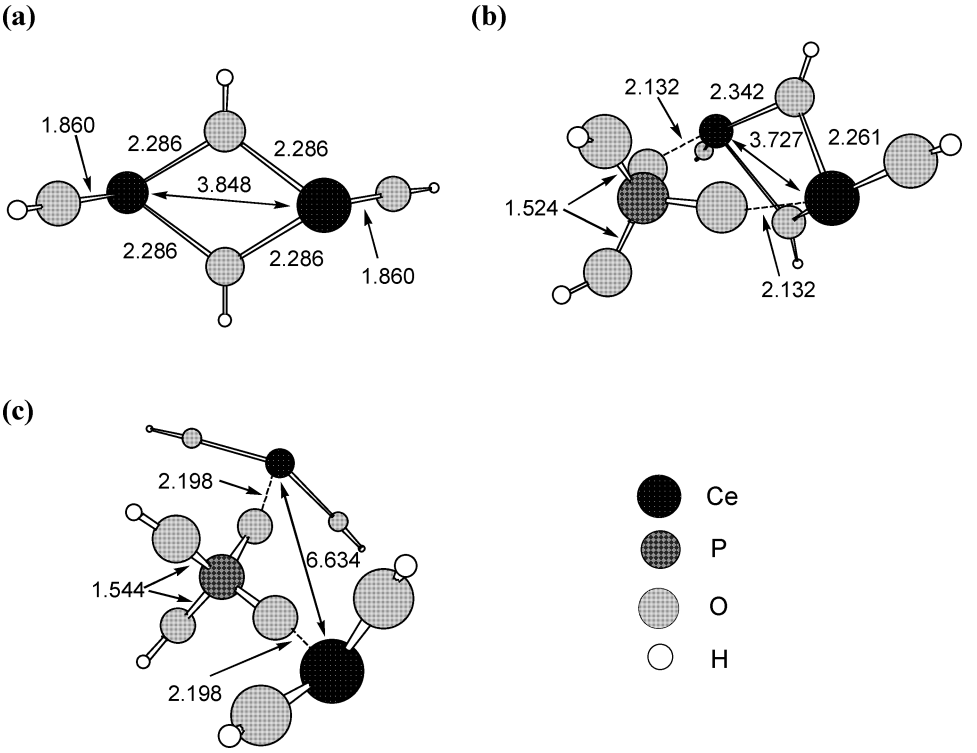


FIGURE 3 The optimized structures determined by the *ab initio* quantum-chemical calculations. (a) $[Ce_2^{IV}(OH)_4]^{4+}$ involving two OH bridges, (b) 1:1 complex between $H_2PO_4^-$ (a model compound of phosphodiester) and $[Ce_2^{IV}(OH)_4]^{4+}$, (c) the ternary complex between $H_2PO_4^-$ and two $[Ce^{IV}(OH)_2]^{2+}$ molecules. The bond length and the interatomic distance are in Å unit.

For the purpose of comparison, the calculation was also carried out on the 1:2 complex between one $H_2PO_4^-$ molecule and two $[Ce^{IV}(OH)_2]^{2+}$ clusters. In the optimized structure of this ternary system, each of the two oxygen atoms of $H_2PO_4^-$ is coordinated to either of these two $[Ce^{IV}(OH)_2]^{2+}$ molecules (Figure 3c). The Ce-O distances are 2.198 Å. The two Ce atoms are sufficiently far away from each other (the distance is 6.634 Å) and thus these two $[Ce^{IV}(OH)_2]^{2+}$ clusters behave as independent species. This 1:2 complex is quite unstable in comparison with the 1:1 complex between $H_2PO_4^-$ and the dinuclear $[Ce_2^{IV}(OH)_4]^{4+}$ cluster in Figure 3b. The free energy change for its formation, evaluated by the calculation, is only –11.04 kcal mol^{-1}, which is far less favorable than the corresponding value (–20.31 kcal mol^{-1}) for the latter complex. The charge of P atom is +2.50, which is by 0.07 unit smaller than the value in the complex of the bimetallic cluster $[Ce_2^{IV}(OH)_4]^{4+}$. It is highly unlikely that this ternary complex is making a significant contribution to the phosphodiester hydrolysis.

Proposed Mechanism of the Hydrolysis of Phosphodiesters

The present kinetic studies have concluded that $[Ce_2^{IV}(OH)_4]^{4+}$ is the active species for the hydrolysis of cAMP and DNA in acidic solutions. Of all the Ce(IV) ions in the mixtures, only less than half is forming this bimetallic hydroxo-cluster (see Table 1: $[Ce^{IV}(OH)_2]^{2+}$ is the most abundant species). Even under these conditions, $[Ce_2^{IV}(OH)_4]^{4+}$ governs the whole reaction. The catalytic activity of this bimetallic cluster must be sufficiently large, in comparison with the activities of the other species. Upon the complex formation of the phosphodiester with this Ce(IV) cluster, its two oxygen atoms are coordinated to each of the two Ce(IV) ions in the cluster (Figure 3b). This bidentate coordination is consistent with the results of previous core-level photoelectron spectroscopy[12] on the complex formation between diphenyl phosphate and Ce(IV). Because of this coordination, the electrons of phosphodiester are greatly withdrawn by the Ce(IV) cluster. The structure of $[Ce_2^{IV}(OH)_4]^{4+}$ is suitable for this complex formation so that the Ce-Ce distance is little altered on the coordination of $H_2PO_4^-$ (3.848 Å → 3.727 Å).

On the basis of these results, the mechanism of the phosphodiester hydrolysis is proposed as depicted in Figure 4.[18] First, the Ce(IV)-bound hydroxide ion attacks the phosphorus atom as the electrophilic center.[19] Since the phosphodiester residue is strongly activated by its bidentate coordination to the two Ce(IV) ions, this intramolecular reaction is efficient. The positive charges in $[Ce_2^{IV}(OH)_4]^{4+}$ stabilize the negatively charged transition-state of the hydrolysis. On the breakdown of the pentacoordinated intermediate, the water bound to the Ce(IV) ions functions as acid catalyst and assists the removal of leaving group from the phosphorus atom. Notable D_2O solvent isotope effect (2.0) observed for this reaction[5a] is probably associated

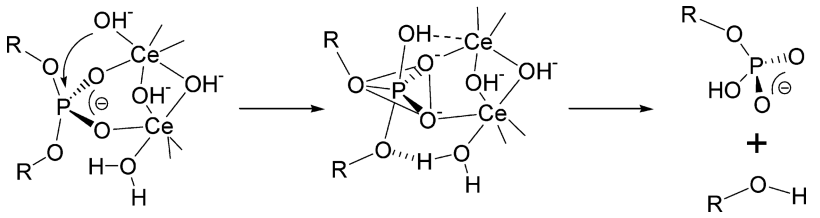


FIGURE 4 Proposed mechanism for the hydrolysis of phosphodiester by bimetallic hydroxo-cluster $[Ce_2^{IV}(OH)_4]^{4+}$.

with the proton-transfer in this process. Consistently, various dinuclear and trinuclear metal complexes are far more active for phosphoester hydrolysis than are the corresponding mononuclear complexes.[20,21] Many enzymes for phosphodiester hydrolysis also involve two or three metal ions at their active center.[22] The proposed mechanism of catalysis is further supported by previous core-level photoelectron spectroscopy[12] showing that the 2p orbital of P atom of diphenyl phosphate in its Ce(IV) complex is far more stable than those in the complexes of diphenyl phosphate with other metal ions. Apparently, the Ce(IV) ion withdraws electrons from the phosphodiester in far greater extent than do the other metal ions, and drastically enhances the electrophilicity of the P atom.[23] The results of quantum-chemical calculation in this article provide strong supports for these arguments.

CONCLUSION

Among various non-enzymatic catalysts hitherto proposed, only Ce(IV) ion can hydrolyze cAMP and DNA at reasonable rates under physiological conditions. The bimetallic hydroxo-cluster $[Ce_2^{IV}(OH)_4]^{4+}$ is the catalytically active species, as shown by the present kinetic analysis on the hydrolysis in acidic solutions. Although the concentration of this cluster is not necessarily large, it governs the reaction due to its large catalytic rate constant. It is proposed that the stable phosphodiester linkages are strongly activated by the cooperation of two Ce(IV) ions in this cluster. According to quantum-chemical theoretical studies, the two Ce(IV) ions at around 3.8 Å distance in this dinuclear Ce(IV) cluster bind two non-bridging oxygen atoms of the phosphodiester linkages, and notably withdraw electrons from this linkage. The electron-withdrawing activity of Ce(IV) is overwhelmingly greater than those of the other metal ions. The phosphorus atom in the phosphodiester linkage thus activated is efficiently attacked by the Ce(IV)-bound hydroxide, resulting in the hydrolytic scission of the linkage. The origin of remarkable catalysis of Ce(IV) for phosphodiester hydrolysis has been for the first time quantitatively clarified. When the hydrolyses are carried out at around pH 7, similar acid-base cooperation should occur in higher

aggregates of $[Ce_2^{IV}(OH)_4]^{4+}$. The results in the present article should be useful for design of still more effective catalysts for biologically important phosphodiesters.[24]

EXPERIMENTAL SECTION

Materials

cAMP and TpT were purchased from Sigma. $Ce(NH_4)_2(NO_3)_6$ was obtained from Nacalai. Water was purified with a Millipore purification system Milli-Q (the specific resistance >18.3 $M\Omega \cdot cm^{-1}$) and sterilized immediately before use. Throughout the experiments, great care was taken to avoid contamination by natural enzymes.

Kinetic Measurements

Typical procedure for the hydrolysis of cAMP and TpT was as follows. To 1.0 mL of 3.0 *M* $NaClO_4$ solution, $Ce(NH_4)_2(NO_3)_6$ was added, and then the pH of mixture was adjusted. The reaction was initiated by adding 10 μl of a stock solution of substrate. The reaction conditions (25°C and $[NaClO_4] = 3.0$ *M*) are the same as those where the equilibrium constants $Q_{x,y}$ in Eqs. (2)–(7) were determined in ref 13. The initial concentration of cAMP or TpT was 0.1 *mM*. At appropriate interval, the reaction mixture was analyzed by the reversed-phase HPLC (Merck LiChrospher 18(e) ODS column, 25 cm). The eluent for the analysis of cAMP hydrolysis was 97:3 mixture containing choline chloride as an ion-pair agent, whereas 93:7 water-acetonitrile mixture was used for TpT hydrolysis. The HPLC peaks were assigned by coinjection with authentic samples. The change in pH during the reactions was less than 0.1 unit. The rate constants for the hydrolysis of cAMP were determined by pseudo-first-order plots. On the other hand, the hydrolysis of TpT at 25°C was slow so that the reaction rate was determined from the initial slope of time-conversion curve. Duplicate or triplicate runs were made and the differences between the rate constants obtained were within 10%. The absence of contamination by enzymes and others was confirmed by repeated control experiments.

Light-Scattering Photometry

The aquesous solutions of $Ce(NH_4)_2(NO_3)_6$ (1 *mM*) at pH 2.0 were analyzed by an Otsuka Electronics dynamic photoscattering FDLS-3000 spectrometer. This analyzer can detect, if any, the particles which are greater than 15 Å.

Determination of the Concentration of Tetravalent Ce Ion in the Reaction Mixtures

In order to confirm the lack of reduction of Ce(IV) to the trivalent ion during the hydrolysis of cAMP and TpT, the concentration of Ce(IV) in the reaction mixtures was directly determined by titration. A given amount of $FeSO_4$ was added to the mixture, and the resultant solution was back-titrated with $Ce(NH_4)_4(SO_4)_4$ using *o*-phenanthroline as an indicator (its Fe(II) complex is red, while the Fe(III) complex is light purple). At least duplicate runs were made for each determination. The solutions of $FeSO_4$ and $Ce(NH_4)_4(SO_4)_4$ were prepared immediately before use.

Quantum-Chemical Calculations

The GAUSSIAN 98 program was used,[25] and the geometries of all the mono- and dinuclear clusters of Ce(IV), free $H_2PO_4^-$ and their complexes were optimized in a gas phase at RHF level. The Gibbs free energies were obtained by the density-functional-theory calculation with B3LYP level. Contribution of the solvation effects was incorporated by the PCM on the recommended conditions.[26] In the geometry optimization, the basis function for the P, O, C, and H atoms was $3-21+G^*$, while $6-31+G^*$ was used in the Gibbs free energy calculation. The effective core potential methods were applied to a 46-electron core ([Kr]4d) of the Ce(IV) atom.[27] The 4f, 5s, and 5p orbitals were included as outer core and calculated by using the basis set size of DZP.

REFERENCES

1. (a) Copeland, K.D.; Fitzsimons, M.P.; Houser, R.P.; Barton, J.K. DNA hydrolysis and oxidative cleavage by metal-binding peptides tethered to rhodium intercalators. Biochemistry **2002**, 41, 343–356 and references cited therein. (b) Komiyama, M.; Sumaoka, J.; Kuzuya, A.; Yamamoto, Y. Sequence-selective artificial ribonucleases. Methods in Enzymology **2001**, 341, 455–468. (c) Sreedhara, A.A.; Cowan, J. Catalytic hydrolysis of DNA by metal ions and complexes. Journal of Biological Inorganic Chemistry **2001**, 6, 337–347. (d) Komiyama, M.; Takeda, N.; Shigekawa, H. Hydrolysis of DNA and RNA by lanthanide ions: mechanistic studies leading to new applications. Chemical Communications **1999**, 1443–1451. (e) Williams, N.H.; Takasaki, B.; Wall, M.; Chin, J. Structure and nuclease activity of simple dinuclear metal complexes: Quantitative dissection of the role of metal ions. Accounts of Chemical Research **1999**, 32, 485–493. (f) Komiyama, M.; Sumaoka, J. Progress towards synthetic enzymes for phosphoester hydrolysis. Current Opinion in Chemical Biology **1998**, 2, 751–757. (g) Hegg, E.L.; Burstyn, J.N. Toward the development of metal-based synthetic nucleases and peptidases: a rationale and progress report in applying the principles of coordination chemistry. Coordination Chemistry Reviews **1998**, 173, 133–165. (h) Trawick, B.N.; Daniher, A.T.; Bashkin, J.K. Inorganic mimics of ribonucleases and ribozymes: From random cleavage to sequence-specific chemistry to catalytic antisense drugs. Chemical Reviews **1998**, 98, 939–960. (i) Oivanen, M.; Kuusela, S.; Lönnberg, H. Kinetics and mechanisms for the cleavage and isomerization of the phosphodiester bonds of RNA by Brønsted acids and bases. Chemical Reviews **1998**, 98, 961–990. (j) Morrow, J.R.; Iranzo, O. Synthetic metallonucleases for RNA cleavage. Current Opinion in Chemical Biology **2004**, 8, 192–200.
2. Recent reviews: (a) ref. 1b. (b) ref. 1h. (c) ref. 1i. (d) ref. 1j. (e) Perreault, D.M.; Anslyn, E.V. Unifying the current data on the mechanism of cleavage—Transesterification of RNA. Angewandte Chemie International Edition **1997**, 36, 432–450.

3. Chin, J.; Zou, X. Catalytic hydrolysis of cAMP. Canadian Journal of Chemistry **1987**, 65, 1882–1884.
4. Chin, J.; Banaszczyk, M.; Jubian, V.; Zou, X. Co(III) complex promoted hydrolysis of phosphate diesters—comparison in reactivity of rigid cis-diaquotetraazacobalt(III) complexes. Journal of the American Chemical Society **1989**, 111, 186–190.
5. (a) Komiyama,M.; Takeda, N.; Takahashi, Y.; Uchida, H.; Shiiba, T.; Kodama, T.; Yashiro, M. Efficient and oxygen-independent hydrolysis of single-stranded-dna by cerium(IV) ion. Perkin Transactions 2 **1995**, 269–274. (b) Komiyama, M.; Shiiba, T.; Kodama, T.; Takeda, N.; Sumaoka, J.; Yashiro, M. DNA hydrolysis by cerium(IV) does not involve either molecular-oxygen or hydrogen-peroxide. Chemistry Letters **1994**, 1025–1028. (c) Komiyama, M.; Kodama, T.; Takeda, N.; Sumaoka, J.; Shiiba, T.; Matsumoto, Y.; Yashiro, M. Catalytically active species for $CeCl_3$-induced DNA hydrolysis. Journal of Biochemistry **1994**, 115, 809–810. (d) Takasaki, B.K.; Chin, J. Cleavage of the phosphate diester backbone of DNA with cerium(III) and molecular-oxygen. Journal of the American Chemical Society **1994**, 116, 1121–1122. (e) Ihara, T.; Shimura, H.; Ohmori, K.; Tsuji, H.; Takeuchi, J.; Takagi, M. Hydrolysis of nucleotides using actinoid metal ion. Chemistry Letters **1996**, 687–688. (f) references cite in ref. 1.
6. Ce(IV) complexes are also active for DNA hydrolysis: (a) Rammo, J.; Hettich, R.; Roigk, A.; Schneider, H-J. Catalysis of DNA cleavage by lanthanide complexes with nucleophilic or intercalating ligands and their kinetic characterization. Chemical Communications **1996**, 105–107. (b) Hashimoto, S.; Nakamura, Y. Characterization of lanthanide-mediated DNA cleavage by intercalator-linked hydroxamic acids: Comparison with transition systems. Perkin Transactions 1 **1996**, 2623–2628. (c) Igawa, T.; Sumaoka, J.; Komiyama, M. Hydrolysis of oligonucleotides by homogeneous Ce(IV)/EDTA complex. Chemistry Letters **2000**, 356–357. (d) Branum, M.E.; Tipton, A.K.; Zhu, S.; Que, L.Jr. Double-strand hydrolysis of plasmid DNA by dicerium complexes at 37°C. Journal of the American Chemical Society **2001**, 123, 1898–1904. (f) Kovacic, R.T.; Welch, J.T.; Franklin, S. J. Sequence-Selective DNA Cleavage by a Chimeric Metallopeptide. Journal of the American Chemical Society **2003**, 125, 6656–6662. (g) references cite in ref. 1.
7. Catalysis by Ce(IV) for cAMP hydrolysis: (a) Sumaoka, J.; Yashiro, M.; Komiyama, M. Remarkably fast hydrolysis of 3′,5′-cyclic adenosine-monophosphate by cerium(III) hydroxide cluster. Journal of the Chemical Society, Chemical Communications **1992**, 1707–1708. (b) Sumaoka, J.; Miyama, S.; Komiyama, M. Enormous acceleration by cerium(IV) for the hydrolysis of nucleoside 3′,5′-cyclic monophosphates at pH 7. Journal of the Chemical Society, Chemical Communications **1994**, 1755–1756. (c) Cullis, P.M.; Snip, E. Stereochemical Course of Cerium(IV)-Catalyzed Hydrolysis of Cyclic Nucleotides. Journal of the American Chemical Society **1999**, 121, 6125–6130.
8. Monophosphates are also hydrolyzed by Ce(IV) ion: Miyama, S.; Asanuma, H.; Komiyama, M. Hydrolysis of phosphomonoesters in nucleotides by cerium(IV) ions. Highly selective hydrolysis of monoester over diester in concentrated buffers. Perkin Transactions 2 **1997**, 1685–1688.
9. In place of using Ce(IV) salts, catalytically active species can be formed by oxidizing Ce(III) ions with molecular oxygen (refs 5b and d). Molecular oxygen is not required for the catalytic process, and thus the DNA hydrolysis by Ce(IV) salts is efficient even in its absence (ref. 5b).
10. Lanthanide(III) ions hydrolyze the phosphate esters other than cAMP and DNA: (a) Hay, R.W.; Govan, N. A lanthanum macrocycle catalyzed-hydrolysis of a phosphate trimester. Journal of the Chemical Society, Chemical Communications **1990**, 714–715. (b) Breslow, R.; Zhang, B.L. Cleavage of phosphate esters by a cyclodextrin dimer catalyst that binds the substrates together with La^{3+} and hydrogen peroxide. Journal of the American Chemical Society **1994**, 116, 7893–7894. (c) Morrow, J.R.; Aures, K.; Epstein, D. Metal-ion promoted attack of an alcohol on a phosphate diester—modeling the role of metal-ions in RNA self-splicing reactions. Journal of the Chemical Society, Chemical Communications **1995**, 2431–2432. (d) Matsumura, M.; Komiyama, M. Enormously fast RNA hydrolysis by lanthanide(III) ions under physiological conditions: Eminent candidates for novel tools of biotechnology. Journal of Biochemistry **1997**, 122, 387–394 and references cited therein.
11. Shigekawa, H.; Ishida, M.; Miyake, K.; Shioda, R.; Iijima, Y.; Imai, T.; Takahashi, H.; Sumaoka, J.; Komiyama, M. Extended x-ray absorption fine structure study on the cerium(IV)-induced DNA hydrolysis: Implication to the roles of 4f orbitals in the catalysis. Applied Physics Letters **1999**, 74, 460–462.
12. Shigekawa, H.; Ikawa, H.; Yoshizaki, R.; Iijima, Y.; Sumaoka, J.; Komiyama, M. Core level photoelectron spectroscopy on the lanthanide-induced hydrolysis of DNA. Applied Physics Letters **1996**, 68, 1433–1435.

13. Base, C.F., Jr.; Mesmer, R.E. 1976. *The Hydrolysis of Cations*, 138–146. John Wiley & Sons, New York.
14. The half-life of cAMP is only 9 min, corresponding to more than 10^{13}-fold acceleration with respect to non-catalyzed reaction.
15. The ratio of adenosine 3′-monophosphate to its 5′-monophosphate was about 10:1. The P-O(5′) linkage was preferentially cleaved.
16. These kinetic analyses were performed on the assumption that only one species of Ce(IV) is responsible for the hydrolytic reaction. The possibility that the combination of Ce(IV)-derived species is active for the reaction can not be completely ruled out. However, all the results presented here point to the overwhelming contribution of the proposed species to the catalysis.
17. In Figure 3, water molecules are omitted because of clarity.
18. This mechanism is different from that proposed for the catalysis by $CeCl_3/O_2$ combination in ref. 5d, although both involve dinuclear Ce(IV) species. In the previously proposed mechanism, the two Ce(IV) ions in the cluster are bridged by -O-O- group, which is derived from molecular oxygen, and this group is the nucleophile for the reaction. Thus, molecular oxygen is inevitable for the catalysis. In the present mechanism, however, the metal-bound hydroxide as the nucleophile comes from water. Consistently, no molecular oxygen is required for the hydrolysis of cAMP and DNA by $Ce(NH_4)_2(NO_3)_6$ (see ref. 9).
19. If the reaction were involving nucleophilic attack by external hydroxide ion in the reaction mixtures, the reaction rate at pH 7 should be as 10^5 times as large as that at pH 2. Apparently, it is not the case.
20. (a) Yashiro, M.; Ishikubo, A.; Komiyama, M. Preparation and study of dinuclear zinc(II) complex for the efficient hydrolysis of the phosphodiester linkage in a diribonucleotide. Journal of the Chemical Society, Chemical Communications **1995**, 1793–1794. (b) Young, M.J.; Chin, J. Dinuclear copper(II) complex that hydrolyzes RNA. Journal of the American Chemical Society **1995**, 117, 10577–10578. (c). Yashiro, M; Ishikubo, A.; Komiyama, M. Efficient and unique cooperation of three zinc(II) ions in the hydrolysis of diribonucleotides by a trinuclear zinc(II) complex. Chemical Communications **1997**, 83–84. (d) Molenveld, P.; Engbersen, J.F.J.; Reinhoudt, D.N.; Specific RNA dinucleotide cleavage by a synthetic calix[4]arene-based trinuclear metallo(II)-phosphodiesterase. Angewandte Chemie International Edition **1999**, 38, 3189–3192. (e) Chapman, W.H. Jr.; Breslow, R. Selective hydrolysis of phosphate esters, nitrophenyl phosphates and UpU, by dimeric zinc complexes depends on the spacer length. Journal of the American Chemical Society **1995**, 117, 5462–5469. (f) Yamada, K.; Takahashi, Y.; Yamamura, H.; Araki, S.; Saito, K.; Kawai, M. Phosphodiester bond cleavage mediated by a cyclic β-sheet peptide-based dinuclear zinc(II) complex. Chemical Communications **2000**, 1315–1316. (g) Gajda, T.; Krämer, R.; Jancsó, A. Structure, equilibrium and ribonuclease activity of copper(II) and zinc(II) complexes formed with a dinucleating bis-imidazole ligand. European Journal of Inorganic Chemistry **2000**, 1635–1644. (h) Worm, K.; Chu, F.; Matsumoto, K.; Best, M.D.; Lynch, V.; Anslyn, E.V. Preorganized bis-zinc phosphodiester cleavage catalysts possessing natural ligands: A lesson pertinent to bimetallic artificial enzymes. Chemistry—A European Journal **2003**, 9, 741–747. (i) Liu, S.; Hamilton, A.D. Rapid and highly base selective RNA cleavage by a dinuclear Cu(II) complex. Chemical Communications **1999**, 587–588. (j) Iranzo, O.; Elmer, T.; Richard, J.P.; Morrow, J.R. Cooperativity between metal ions in the cleavage of phosphate diesters and RNA by dinuclear Zn(II) catalysts. Inorganic Chemistry **2003**, 42, 7737–7746. (k) Liu, C.; Wang, M.; Zhang, T.; Sun, H. DNA hydrolysis promoted by di- and multi-nuclear metal complexes. Coordination Chemistry Reviews **2004**, 248, 147–168.
21. The RNA hydrolysis by lanthanide(III) ions was also ascribed to the catalysis by bimetallic hydroxo-clusters: ref. 10d.
22. Sträter, N.; Lipscomb, W.N.; Klabunde, T.; Krebs, B. Two-metal ion catalysis in enzymatic acyl- and phosphoryl-transfer reactions. Angewandte Chemie International Edition **1996**, 35, 2024–2055.
23. The stability of the trivalent state of Ce should be favorable for this electron-removal. Among all the lanthanide metals, only Ce takes stable tetravalent state in addition to stable trivalent state.
24. Quite recently, site-selective scission of DNA by Ce(IV)/EDTA complex in which monophosphate groups of oligonucleotide additives recruit the Ce(IV) to the target site was reported (Chen, W.; Kitamura, Y.; Zhou, J.-M.; Sumaoka, J.; Komiyama, M. Site-selective DNA hydrolysis by combining Ce(IV)/EDTA with monophosphate-bearing oligonucleotides and enzymatic ligation of the scission fragments. Journal of the American Chemical Society **2004,** 126, 10285–10291). The information presented here should be useful to understand the mechanism of these artificial restriction enzymes.
25. Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Zakrzewski, V.G.; Montgomery, J.A.; Stratmann, J.C. Burant, S.; Dapprich, J.M.; Millam, A.D.; Daniels, K.N.;

Kudin, M.C.; Strain, R.E.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G.A; Ayala, P.Y.; Cui, Q.; Morokuma, K.; Malick, D.K.; Rabuck, A.D.; Raghavachari, K.; Foresman, J.B.; Cioslowski, J.; Ortiz, J.V.; Baboul, A.G.; Stefanov, B.B.; Liu, G.; Liashenko, A.; Piskorz, I.; Komaromi, R.; Gomperts, R.L.; Martin, D.J.; Fox, T.; Keith, M.A.; Al-Laham, P.; Peng, C.Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P.M.W.; Johnson, B.G.; Chen, W.; Wong, M.W.; Andres, J.L.; Gonzalez, C.; Head-Gordon, M.; Replogle, E.S.; Pople, J.A. Gaussian 98 (Rev. A.7), Gaussian, Inc., Pittsburgh, PA, 1998.

26. Solvent, water; Model, PCM/UAHF; Icomp = 4; Version, Matrix inversion; Cavity, Pentakisdodecahedra with 60 initial tesserae. Barone, V.; Cossi, M.; Tomasi, J. A new definition of cavities for the computation of solvation free energies by the polarizable continuum model. The Journal of Chemical Physics **1997**, 107, 3210–3221.
27. Cundari, T.R.; Stevens, W.J. Effective core potential study of transition-metal and lanthanide catalyzed hydrogen-exchange. The Journal of Chemical Physics **1993**, 98, 5555–5565.