Communications to the Editor

Cleavage of the Phosphate Diester Backbone of DNA with Cerium(III) and Molecular Oxygen

Bryan K. Takasaki and Jik Chin*

Department of Chemistry, McGill University
Montreal, Canada H3A 2K6

Received September 28, 1993

Synthetic nucleases which rapidly cleave nucleic acids under mild conditions have many important potential applications ranging from the synthesis of custom-designed artificial restriction enzymes[1] to the development of new antitumor agents.[2] Catalysts that cleave DNA oxidatively by generating hydroxyl radicals have been used in many innovative studies;[3,4] however, they modify the deoxyribose moiety, producing fragments which cannot be religated.[5] Furthermore, the diffusable hydroxyl radicals can produce multiple cleavage sites. Hydrolytic cleavage suffers from neither of these drawbacks. Although considerable progress has been made in the development of catalysts capable of hydrolytic cleavage of unactivated phosphate diesters, these systems still cleave phosphate diesters much more slowly than natural enzymes and catalysts capable of oxidative cleavage.[6] Here we report that the dinucleotide dApdA (2′-deoxyadenyl(3′–5′)-2′-deoxyadenosine) is rapidly cleaved to give dA (2′-deoxyadenosine) and inorganic phosphate in the presence of Ce(III) and molecular oxygen with no destruction of the deoxyribose moiety.

Conversion of dApdA to dA was monitored by HPLC[7] (Figure 1). The half-life for the cleavage of dApdA (1 mM) with added $Ce(ClO_4)_3$ (20 mM) at pH 8.2 and 37 °C is approximately 2 h. 3′-dAMP (2′-deoxyadenosine 3′-monophosphate) and 5′-dAMP (2′-deoxyadenosine 5′-monophosphate) also undergo rapid cleavage under the above conditions, and their concentrations do not accumulate appreciably during the course of dApdA cleavage[8] (Figure 1). The pseudo-first-order rate constant of dApdA cleavage was obtained by following the initial rate of decrease in the concentration of dApdA ($k_{obs} = 6.8 \times 10^{-3}$ min^{-1}).

None of the other lanthanide ions tested (La, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Er, Tm, Yb) showed any detectable cleavage of dApdA after 68 h under the same conditions used for Ce(III)-mediated cleavage of dApdA. Interestingly, when molecular oxygen is removed from the Ce(III) reaction medium by degassing the solvent with nitrogen gas, no cleavage of dApdA is detected (Figure 2). This observation combined with the fact that cerium is the only lanthanide which can be oxidized to the tetravalent state under the reaction conditions suggests that the mechanism of cleavage is oxidative rather than hydrolytic and may involve the activation of molecular oxygen by Ce(III). Interestingly, the HPLC trace shows only the products expected from the cleavage of the P–O bond of dApdA with no trace of products due to cleavage of the sugar or depurination (adenine elutes from the column at 2.54 min). Analysis of the concentrations of dApdA, dA, 3′-dAMP, and 5′-dAMP as a function of time indicates that all of the reacted dApdA is converted cleanly to dA and the monoesters.[8] Furthermore, inorganic phosphate is the only product detected by ^{31}P NMR.[8]

To test the hypothesis that hydrogen peroxide might be the activated oxygen species involved in the $Ce(III)/O_2$-mediated cleavage of dApdA, we looked for cooperativity between hydrogen peroxide and La(III) in the cleavage of a phosphate diester.[9] Hydrogen peroxide alone (20 mM) does not appreciably increase

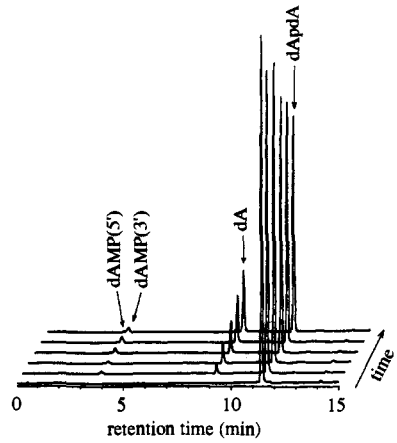


Figure 1. HPLC chromatograms showing the cleavage of dApdA (1 mM) by $Ce(ClO_4)_3$ (20 mM), pH 8.2 at 37 °C. Chromatograms are taken of the reaction mixture at 10-min intervals from 0 to 50 min after mixing of the reactants.

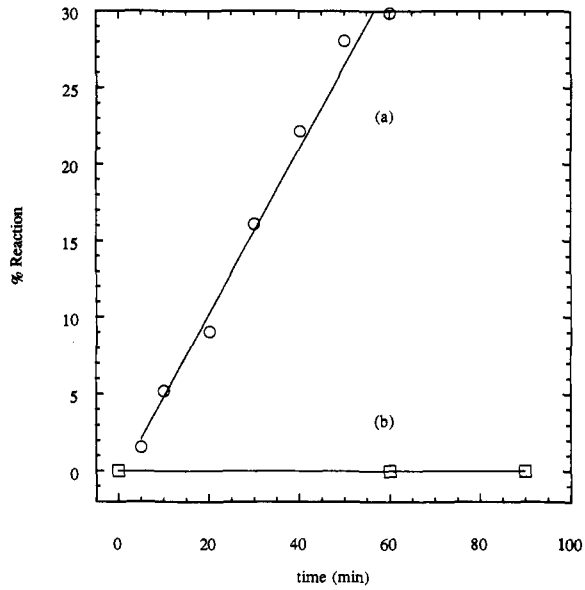
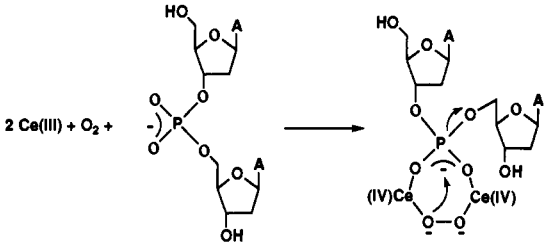


Figure 2. Kinetic plot showing the rate of dApdA (1 mM) cleaved by $Ce(ClO_4)_3$ (20 mM), pH 8.2 at 37 °C: (a) in the presence of atmospheric oxygen; (b) after degassing with nitrogen gas.

(1) Corey, D. R.; Schultz, P. G. *Science* **1987**, *238*, 1401–1403.
(2) Nicolaou, K. C.; Dai, W.-M. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1387–1530.
(3) Strobel, S. A.; Dervan, P. B. *Science* **1990**, *249*, 73–75.
(4) Sigman, D. S.; Bruice, T. W.; Mazumder, A.; Sutton, C. L. *Acc. Chem. Res.* **1993**, *26*, 98–104.
(5) It has recently been shown that the damage to the sugars caused by hydroxylation of the C–H bond at the 5′-position can be reversed by treatment with $NaBH_4$. Pratviel, G.; Durarte, V.; Bernadou, J.; Meunier, B. *J. Am. Chem. Soc.* **1993**, *115*, 7939–7943.
(6) (a) Kim, J. H.; Chin, J. *J. Am. Chem. Soc.* **1992**, *114*, 9792–9795. (b) Chin, J. *Acc. Chem. Res.* **1991**, *24*, 145.
(7) In a typical run, 50 μL of the reaction mixture was removed and quenched with 50 μL of 0.2 M inorganic phosphate (pH 5.5). The cerium phosphate precipitate was spun down for 1 min at 10 000 rpm; 1 μL of the supernatant was then injected onto a C-18 reversed-phase column (5-μm Hypersil maintained at 40 °C) and eluted for 5 min with $NH_4H_2PO_4$ (0.2 M, pH 5.5) followed by a 0–50% linear gradient of $NH_4H_2PO_4$ (0.2 M, pH 5.5) and methanol/water (3:2) solutions over 10 min with a flow rate of 0.5 mL/min.
(8) See supplementary material: (a) Figure S1 for HPLC chromatograms and summary of results for $Ce(III)$-O_2-mediated cleavage of 3′-dAMP and 5′-dAMP; (b) Figure S2 for graph showing the concentration of dApdA, dAMP, and dA as a function of time; (c) Figure S3 for ^{31}P NMR due to cleavage of dApdA.
(9) Takasaki, B. K.; Chin, J. *J. Am. Chem. Soc.* **1993**, *115*, 9337–9338.

0002-7863/94/1516-1121$04.50/0

Scheme 1 Proposed Mechanism for the Ce(III)/O_2-Mediated Cleavage of dApdA



the rate of hydrolysis of BNPP (bis(*p*-nitrophenyl) phosphate). However, when $La(ClO_4)_3$ (2 mM) is combined with hydrogen peroxide (20 mM), the rate of BNPP hydrolysis increases by (3.4×10^4)-fold over the rate in the presence of La(III) alone. Furthermore, the pH dependence data suggests that the active form of the catalyst is a La(III) peroxide dimer formed from two La(III) ions and two peroxide dianions.[9]

While we were investigating the mechanism of dApdA cleavage, it was reported by Komiyama *et al.* that Ce(III) hydrolyzes the DNA dinucleotide (dTpdT) by intramolecular metal hydroxide attack on the coordinated phospahte diester.[10] However, this mechanism does not explain the oxygen dependence of the reaction, nor does it account for the enormous difference in reactivity between Ce(III) and the other lanthanide ions.[11] Lanthanide ions have previously been shown to vary only slightly in their reactivity toward RNA and RNA analogs.[12]

One possible mechanism for the dApdA cleavage which accounts for all of the data and is analogous to the mechanism proposed for the La(III) peroxide cooperativity involves the oxidation of Ce(III) to Ce(IV) by molecular oxygen followed by intramolecular Ce peroxide attack on the coordinated phosphate diester (Scheme 1). In the first step two Ce(III) ions are oxidized to Ce(IV) by molecular oxygen, giving rise to a peroxide dianion coordinated to two Ce(IV) ions.[13] In the second step the Ce peroxide attacks the coordinated phosphate diester, releasing the 2′-deoxyadenosine product. No peroxyphosphate intermediate was detected. The peroxide group in the Ce(III)-coordinated peroxyphosphate should be a good leaving group and easily displaced by the solvent.

This mechanism combines the effects of Lewis acid activation of the phosphate by coordination to the metal ions[6] with intramolecular nucleophilic attack by the coordinated peroxide anion.[14] It was recently shown that hydrogen peroxide can act as an efficient nucleophile for cleaving biologically important polymers. It has been reported that cooperativity between the Flp recombinase and hydrogen peroxide results in the site-specific cleavage of DNA.[15] Furthermore, a mechanism involving nucleophilic attack by metal-bound peroxide has been proposed not only for the cleavage of a phosphate diester[9] but also for the cleavage of amides such as DMF[16] and the amide linkage in protein molecules.[17]

In conclusion, facile oxidative cleavage of the dApdA phosphate diester bond can be achieved with Ce(III) and molecular oxygen. Unlike previously reported catalysts that cleave DNA oxidatively, the deoxyribose ring is not damaged during the cleavage reaction.

Acknowledgment. Financial support was provided by the Natural Sciences and Engineering Research Council of Canada and the Respiratory Health Networks of Centers of Excellence. B.T. gratefully acknowledges a postgraduate fellowship from N.S.E.R.C.

Supplementary Material Available: Figures S1, S2, and S3 as described in footnote 8 (3 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(10) Komiyama, M.; Matsumura, K.; Yonezawa, K.; Matsumoto, Y. *Chem. Express* **1993**, *8*, 85–8.

(11) Komiyama and co-workers reported that the phosphate diester bond of 3′,5′-cAMP is cleaved by Ce(III) almost 10^3 times faster than any of the other lanthanides. Sumaoka, J.; Yashiro, M.; Komiyama, M. *J. Chem. Soc., Chem. Commun.* **1992**, 1707–1708.

(12) (a) Morrow, J. R.; Buttrey, L. A.; Berback, K. A. *Inorg. Chem.* **1992**, *31*, 16–20. (b) Morrow, J. R.; Buttrey, L. A.; Shelton, V. M.; Berback, K. A. *J. Am. Chem. Soc.* **1992**, *114*, 1903–1905. (c) Breslow, R.; Huang, D.-L. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4080.

(13) Although the reduction potential of Ce(IV) in acid medium is high (1.4–1.6 V), it is strongly dependent on the reaction medium, and under certain conditions, Ce(III) can be oxidized to Ce(IV) by mild oxidants such as benzoquinone (E = 0.650 V). Sen, A.; Stecher, H. A.; Rheingold, A. L. *Inorg. Chem.* **1992**, *31*, 473–479.

(14) Larsson, L. *Acta Chem. Scand.* **1958**, *12*, 723–730.

(15) Kimball, A. S.; Lee, J.; Jayaram, M.; Tullius, T. D. *Biochemistry* **1993**, *32*, 4698–4701.

(16) Murthy, N. N.; Mahroof-Tahir, M.; Karlin, K. D. *J. Am. Chem. Soc.* **1993**, *115*, 10404–10405.

(17) Rana, T. M.; Meares, C. F. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10578–10582.