Synergistic Effect between La(III) and Hydrogen Peroxide in Phosphate Diester Cleavage

Bryan K. Takasaki and Jik Chin*

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

Received May 24, 1993

Over the years there has been a great deal of interest in developing artificial hydrolytic metalloenzymes that hydrolyze the phosphate diester bonds in nucleic acids and the amide bonds in protein molecules. Lanthanide metal ions and their complexes have recently been shown to be highly reactive for hydrolyzing phosphate diesters including RNA.[1] Sequence-specific hydrolytic cleavage of the amide bond in protein molecules through cooperative interactions between metal complexes and hydrogen peroxide has been achieved even though the mechanism of this remarkable reaction is still unclear.[2] In an interesting recent study, Flp recombinase–peroxide cooperativity in cleaving the phosphate diester bond of DNA has been reported.[3] Here we report La(III)–peroxide cooperativity in phosphate diester cleavage.

The pseudo-first-order rate constants for $La(ClO_4)_3$ (2 mM)-promoted cleavage of BNPP (bis(*p*-nitrophenyl)phosphate) with and without hydrogen peroxide at pH 7.0 (50 mM HEPES), 25 °C, were determined by monitoring the increase in visible absorbance at 400 nm due to *p*-nitrophenoxide formation. Cleavage of BNPP gave 2 equiv of nitrophenol. The progress of BNPP cleavage was also monitored by ^{31}P NMR. During the course of the reaction, there are three ^{31}P NMR signals corresponding to inorganic phosphate (δ 0.77), *p*-nitrophenyl phosphate (δ −1.14), and BNPP (δ −10.57).[4] At the end of the reaction, only the signal due to inorganic phosphate remains. As shown in Table I, La(III) (2 mM) alone provides a 1.3×10^4-fold increase in the rate of cleavage of BNPP, while a mixture of La(III) (2 mM) and hydrogen peroxide (20 mM) provides an additional 3.4×10^4-fold rate enhancement, resulting in a total of 4.4×10^8-fold rate enhancement over the background rate. Hydrogen peroxide (20 mM) alone at the same temperature and pH gave no detectable hydrolysis of BNPP after 24 h.

Aqueous $La(ClO_4)_3$ yields no titratable protons at neutral pH. However, when 1 equiv or more of hydrogen peroxide is mixed with the $La(ClO_4)_3$ solution, 2 equiv of protons are released per equivalent of La(III) at neutral pH (Figure 1). The simplest reaction scheme that accounts for the number of protons released involves the formation of a lanthanum peroxide monomer (Scheme Ia). However, the steepness of the titration curve indicates that at least a dimer (Scheme Ib) or higher order aggregates are formed.

Figure 1 shows the titration data with calculated curves fit according to Schemes Ia and Ib using eqs 1a and 1b, respectively,

Table I. Relative Rates of Hydrolysis of BNPP[a]

catalyst	k_{obs} (s^{-1})	relative rate
none[b]	1.1×10^{-11}	1.0
2 mM La(III)	1.4×10^{-7}	1.3×10^4
2 mM La(III) + 20 mM HOOH	4.8×10^{-3}	4.4×10^8

[a] pH 7.0, 50 mM HEPES buffer, 25 °C. [b] Reference 8b.

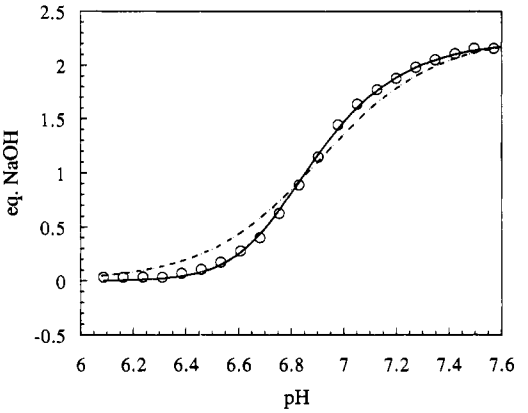
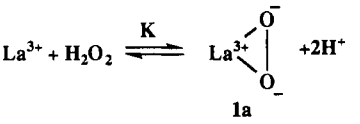


Figure 1. Potentiometric titration of 2 mM $La(ClO_4)_3$ in 0.1 M $NaClO_4$ at 25 °C. (- - -) Calculated curve derived from Scheme Ia. (—) Calculated curve derived from Scheme Ib.

Scheme I

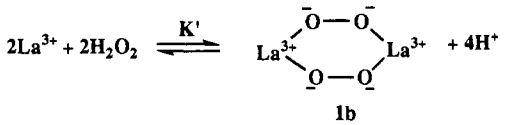
a) monomer:



1a

$$K = \frac{[\mathbf{1a}][H]^2}{[La][H_2O_2]}$$

b) dimer:



1b

$$K' = \frac{[\mathbf{1b}][H]^4}{[La]^2[H_2O_2]^2}$$

where $[OH]_t$ represents the concentration of OH^- consumed, $[La]_t$ represents total La concentration, and K and K' are defined in scheme I.

$$[OH]_t = 2[\mathbf{1a}] = \frac{2K[H_2O_2][La]}{[H]^2 + K[H_2O_2]} \quad (1a)$$

$$[OH]_t = 4[\mathbf{1b}] = ([H]^4 + 4[La]_tK'[H_2O_2]^2 - [H]^2([H]^4 + 8[La]_tK'[H_2O_2]^2)^{1/2})/2K'[H_2O_2]^2 \quad (1b)$$

In an aqueous solution of $La(ClO_4)_3$ (2 mM) and H_2O_2 (20 mM), the rate of cleavage of BNPP increases with increasing pH and levels off at neutrality. Figure 2 shows the pH–rate profile data with the calculated curves fit according to eqs 2a and 2b, which were derived from Scheme I assuming that **1a** or **1b** is the active form.

$$k_{obs} = \frac{kK[H_2O_2][La]}{[H]^2 + K[H_2O_2]} \quad (2a)$$

(1) (a) Morrow, J. R.; Buttrey, L. A.; Shelton, V. M.; Berback, K. A. *J. Am. Chem. Soc.* **1992**, *114*, 1903–1905. (b) Morrow, J. R.; Buttrey, L. A.; Berback, K. A. *Inorg. Chem.* **1992**, *31*, 16–20. (c) Breslow, R.; Huang, D.-L. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4080. (d) Komiyama, M.; Matsumara, K.; Matsumoto, Y. *J. Chem. Soc., Chem. Commun.* **1992**, 640–641. (e) Sumaoka, S.; Yashiro, M.; Komiyama, M.; *J. Chem. Soc., Chem. Commun.* **1992**, 1707–1708.

(2) (a) Rana, T. M.; Meares, C. F. *J. Am. Chem.* **1990**, *112*, 2457–2458. (b) Rana, T. M.; Meares, C. F. *J. Am. Chem. Soc.* **1991**, *113*, 1859–1861. (c) Rana, T. M.; Meares, C. F. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 10578–10582.

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

(4) ^{31}P shifts referenced to 85% H_3PO_4. 5 mM $La(ClO_4)_3$, 50 mM hydrogen peroxide, and 2 mM BNPP were mixed in 0.1 M HEPES buffer (pH 7.0). The insoluble $LaPO_4$ salt formed during the reactions was redissolved by adding a 10-fold excess of EDTA prior to acquisition of the NMR spectrum.

0002-7863/93/1515-9337$04.00/0

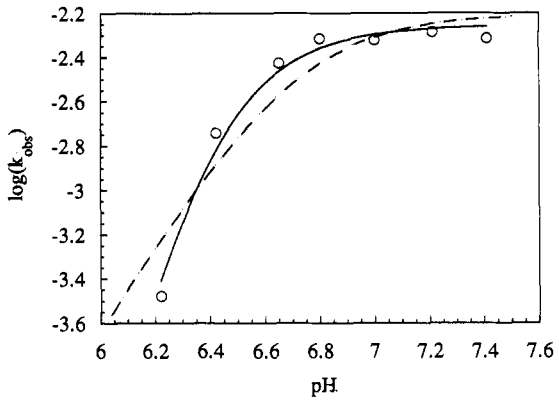
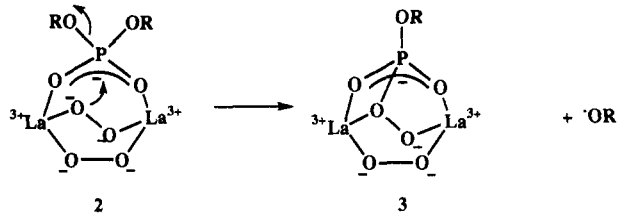


Figure 2. pH–rate profile of BNPP cleavage in the presence of 2 mM $La(ClO_4)_3$, 20 mM HOOH, 50 mM HEPES buffer, 25 °C. (- - -) Calculated curve derived from Scheme Ia. (—) Calculated curve derived from Scheme Ib.

$$k_{obs} = k([H]^4 + 4[La]_t K'[H_2O_2]^2 - [H]^2([H]^4 + 8[La]_t K'[H_2O_2]^2)^{1/2})/8K'[H_2O_2]^2 \quad (2b)$$

It is apparent from the curve fits shown in Figures 1 and 2 that Scheme Ib provides the better fit to both the titration and the pH–rate profile data. This suggests that the dimeric La(III) peroxide complex **1b** is the active species for cleaving BNPP. The value of the equilibrium constant for formation of the complex (K′) obtained from fitting the titration curve (1.4×10^{-23} M) is in good agreement with that obtained from fitting the pH–rate profile (6.6×10^{-22} M).

A possible mechanism for **1b**-catalyzed cleavage of BNPP involves intramolecular La-peroxide attack on the singly or doubly coordinated phosphate diester **2**. Although hydroxide and peroxide have been observed to cleave the P–O and C–O bonds



2 3

of methyl (2,4-dinitrophenyl)phosphate,[5] nucleophilic attack of ethyl (*p*-nitrophenyl)phosphate occurs exclusively at phosphorus.[6] The proposed mechanism for conversion of **2** to **3** involves oxidative cleavage of the phosphate diester bond, resulting in net hydrolytic cleavage of BNPP since peroxyphosphates **3** reduce to phosphates in the presence of hydrogen peroxide.[7]

In conclusion, this study represents the first example of cooperativity between a metal ion and hydrogen peroxide in the hydrolysis of a phosphate diester. Furthermore, the reactivity of **1b** for hydrolyzing BNPP is about 10 times that of $[Co(trpn)(H_2O)_2]^{3+}$, which is the most reactive Co(III) complex reported to date for cleaving the diester (trpn = tris(aminopropyl)amine).[8]

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

Supplementary Material Available: Derivations of equations (2 pages). Ordering information is given on any current masthead page.

(5) Kirby, A. J.; Younas, M. *J. Chem. Soc. B* **1970**, 1165.
(6) Morrow, J. R.; Trogler, W. C. *Inorg. Chem.* **1988**, *27*, 3387.
(7) (a) Epstein, J.; Demek, M. M.; Rosenblatt, D. H. *J. Org. Chem.* **1956**, *21*, 796–798. (b) Larsson, L. *Acta Chem. Scand.* **1958**, *12*, 723–730.
(8) (a) Chin, J. *Acc. Chem. Res.* **1991**, *24*, 145. (b) Chin, J.; Banaszcyk, M.; Jubian, V.; Zou, X. *J. Am. Chem. Soc.* **1989**, *111*, 186.