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Remarkable (~10^{13}) Rate Enhancement in Phosphonate Ester Hydrolysis Catalyzed by Two Metal Ions

Akira Tsubouchi and Thomas C. Bruice*

Department of Chemistry
University of California at Santa Barbara
Santa Barbara, California 93106

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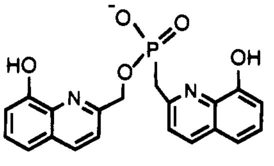
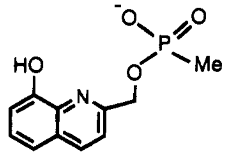
Two metal ions are involved in the catalysis of hydrolysis of phosphate ester bonds by a number of enzymes. The enzymes include the Klenow fragment of polymerase I of *Escherichia coli*,[1] the alkaline phosphatase of *E. coli*,[2] phospholipase C from *Bacillus cereus*,[3] and RNase H from HIV reverse transcriptase.[4] The two metal ions have been considered to cooperate in catalysis. A favored mechanism involves the lowering of the pK_a of a water molecule ligated to one metal ion to provide a ligated hydroxide nucleophile. The second metal ion is then envisioned as being in a position to associate with both the phosphate ester negative charge and the incipient oxyanion leaving group. Examples of catalysis of hydrolysis of a number of substrate types by HO^- ligated by a metal ion[5] or by a metal ion acting to stabilize the pentacoordinate intermediate (transition state)[6] and leaving group[7] are known. We report here the first example, in an intracomplex model, of marked catalysis of the hydrolysis of an aliphatic phosphonate ester brought about by two metal ions acting in concert.[8]

The phosphonate esters **1** and **2** were synthesized from commercially available 8-hydroxy-2-methylquinoline.[9] There are two different structures which represent metal complexes with **1**. The first involves ligation of a single metal ion by both 8-hydroxyquinoline moieties of **1**. Such 1:1 complexes are formed with Ni^{2+}, Co^{2+}, Zn^{2+}, Cu^{2+}, and Al^{3+}. The second involves ligation of two metal ions, one with each 8-hydroxyquinoline moiety. Ester **1** ligates two La^{3+} species. The metal ions Ni^{2+}, Co^{2+}, Zn^{2+}, Cu^{2+}, and Al^{3+} are not catalysts for the hydrolysis of **1**; however, La^{3+} is a very effective catalyst. Inspection of the structure of the 1:2 complex of **1** and La^{3+} (Figure 1) shows that the structural coordinates are ideal for intracomplex catalysis of hydrolysis of the ester P−O bond (see Figure 1 caption). We report now our preliminary studies of the catalysis of the hydrolysis of **1** by La^{3+}.



1



2

Complexation of **1** (2.0×10^{-4} M) with metal ions in water [pH 7.1 (0.1 M HEPES buffer); 30 °C ($\mu = 1.0$ with KCl)] was followed by monitoring the increase in absorbance at 264 nm with increasing metal ion concentration. The data points of Figure 2 have been fitted by a theoretical curve generated from eq 1, which, in turn, is based on the equilibria of Scheme 1 (where $\mathbf{1}_{\text{total}} = EH_2^- + EH^{2-} + E^{3-}$).

Scheme 1

$$EH_2^- + La^{3+} \overset{K_{m1}}{\rightleftharpoons} EHLa^+ + H^+$$

$$EHLa^+ + La^{3+} \overset{K_{m2}}{\rightleftharpoons} ELa_2^{3+} + H^+$$

$$K_{m1} = [EHLa^+]a_H/[EH_2^-][La^{3+}]; \quad K_{m2} = [ELa_2^{3+}]a_H/[EHLa^+][La^{3+}]$$

(E^{3-}: completely deprotonated form of **1**)

$$A_{264} = \frac{A_0 a_H^{\,2} + A_1 a_H K_{m1}[La^{3+}] + A_2 K_{m1} K_{m2}[La^{3+}]^2}{a_H^{\,2} + a_H K_{m1}[La^{3+}] + K_{m1} K_{m2}[La^{3+}]^2} \quad (1)$$

Constants employed in the generation of the best fit are as follows: A_1, 0.746 M^{-1} cm^{-1}; A_2, 1.14 M^{-1} cm^{-1}; K_{m1}, 1.50×10^{-3}; and K_{m2}, 2.43×10^{-6}. In eq 1, A_0 (0.123 M^{-1} cm^{-1}) is the absorbance of EH_2^- when $[La^{3+}] = 0$, A_1 and A_2 refer to the absorbances of $EHLa^+$ and ELa_2^{3+}, respectively, at the initial concentration (2.0×10^{-5} M) of **1**, and K_{m1} and K_{m2} are defined in Scheme 1. The UV−vis spectrum of 8-hydroxy-2-methylquinoline shows no significant change in the presence of La^{3+} beyond 5.0×10^{-3} M, whereas, at this concentration, the phosphonate **2** is completely converted to the 1:1 complex of La^{3+} with **2**.[10] These observations are in accord with the negative charge of the $>PO_2^-$ moiety of **1** and **2** interacting with La^{3+}.

Hydrolysis of **1** in the presence of La^{3+} was followed by HPLC[11] (30 °C, $\mu = 1.0$ with KCl) at constant pH (held constant by HEPES buffer between 6.5 and 7.8). The reaction proceeds with release of (8-hydroxy-2-quinolyl)methanol. Pseudo-first-order rate constants (k_{obsd}, s^{-1}) were obtained from plots of decreasing peak area of **1** *vs* time by fitting the data points to the appropriate equation for the first-order rate law. No buffer catalysis was observed.

A plot of log k_q *vs* pH is provided in the inset to Figure 2. The data points were fitted by use of eq 3, which follows from Scheme 2. Nonlinear least squares fitting provides values of $k_1 = 1.36 \times 10^{-3}$ s^{-1} and p$K_a = 7.19$.

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[8] Recently reported are dinuclear La^{3+} and dinuclear Cu^{2+} promoted hydrolysis of phosphate diesters with a 4-nitrophenol leaving group. (a) Takasaki, B. K.; Chin, J. *J. Am. Chem. Soc.* **1993**, *115*, 9337. (b) Wall, M.; Hynes, R. C.; Chin, J. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1633. (c) Wahnon, D.; Hynes, R. C.; Chin, J. *J. Chem. Soc., Chem. Commun.* **1994**, 1441.

[9] Complete synthetic details will be provided in our full paper. ^1H NMR, IR, and mass spectral data and elemental analysis of **1** and **2** are as required by the assigned structure.

[10] The absorbance at 260 nm increases with increasing $[La^{3+}]$ and levels off at $[La^{3+}] = 5.0 \times 10^{-3}$ M. The data points of the plot of A *vs* $[La^{3+}]$ can be fitted to a theoretical equation assuming single metal binding to **2**.

[11] In a typical kinetic experiment, 4 μL of a stock solution of the phosphonates (**1**, 5.0×10^{-3} M in methanol; **2**, 1.0×10^{-2} M in water) was added to 1 mL of a $LaCl_3$ solution buffered with 0.1 M HEPES. Periodically 20 μL of the reaction mixture was injected onto a C-18 reverse-phase column and eluted with water−methanol (**1**, 42:58; **2**, 55:45, v/v) containing EDTA (5.0×10^{-3} M) and phosphate buffer (5.0×10^{-3} M, pH 4.0) at a flow rate of 1.5 mL/min. The detector wavelength was chosen to be 254 nm for **1** and 310 nm for **2**.

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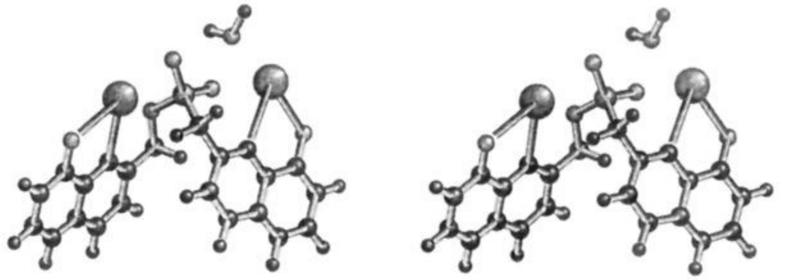


Figure 1. Stereoview of a model of the complex of **1** with two La^{3+}. The La^{3+} to quinoline N bond length (2.70 Å) and the La^{3+} to 8-hydroxyquinoline phenolic oxygen bond length (2.60 Å) have been taken from the literature.[14] The distances from La^{3+} to ligated water and phosphonate oxygens are ideal for catalysis. Thus, the water oxygen, located at the proper distance[15] (2.48 Å) for ligation to La^{3+}, is essentially in line with the leaving group and is located 2.76 Å from P. The second La^{3+} is but 2.65 Å from the departing O. The first and second La^{3+} are located 2.50 and 2.54 Å, respectively, from the two partially negatively charged $>PO_2^-$ oxygens. The structure of the complex of **1** with two La^{3+} was generated in the following manner. The X-ray structure of the complex of Zn^{2+} with 8-hydroxyquinoline was transferred from the Cambridge structural data base and connected to each end of a QUANTA-generated $-CH_2(PO_2^-)OCH_2-$ portion. Zn^{2+} was replaced by La^{3+} and the La−O and La−N bond distances adjusted to proper lengths. The model was generated on a Silicon Graphics 4D/340GTX workstation with the graphics program QUANTA 4.0 (Molecular Simulations, Waltham, MA). The picture was made using Sybyl.

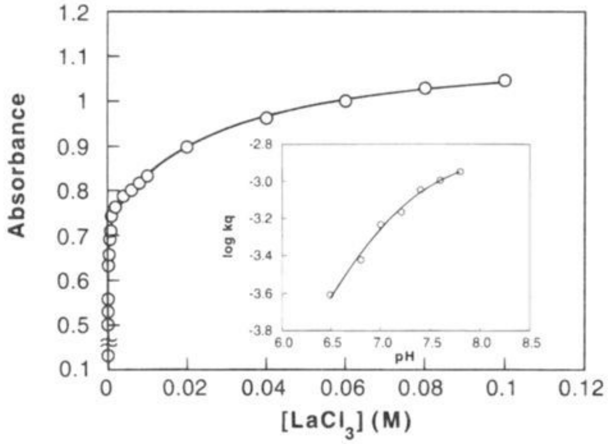


Figure 2. Plot of absorbance (264 nm) of **1** (2.0×10^{-5} M) vs [La^{3+}] at pH 7.1 and 30 °C (μ = 1.0 M with KCl). The solid line was generated by use of eq 1 (Scheme 1). Inset: the pH−rate profile for the spontaneous hydrolysis (k_q) of the 1:2 complex (**1**)La$_2$ at 30 °C (μ = 1.0 M with KCl). The curve was created according to eq 3.

Scheme 2

$$\mathrm{ELa_2}^{3+} \xrightarrow{k_q} \mathrm{P} \tag{2}$$

$$\mathrm{ELa_2}^{3+}(\mathrm{H_2O})_n \underset{+\mathrm{H}^+}{\overset{K_a}{\underset{-\mathrm{H}^+}{\rightleftharpoons}}} \mathrm{ELa_2}^{2+}(\mathrm{H_2O})_{n-1}(\mathrm{HO}^-) \xrightarrow{k_1} \mathrm{P}$$

$$k_q = \frac{k_1 K_a}{K_a + a_H} \tag{3}$$

As shown in Figure 1 and described in the figure caption, the two La^{3+} species in the $ELa_2^{2+}(H_2O)_{n-1}(HO^-)$ allow optimum opportunity for one La^{3+} to associate with both the negative charge on $-(PO_2^-)-$ and the departing incipient $-CH_2O^{\delta-}$ leaving group; the second La^{3+} is ligated to the negative charge on $-(PO_2^-)-$ and an HO$^-$ which is in alignment for a virtual in-line nucleophilic attack on P. We propose this mechanism.

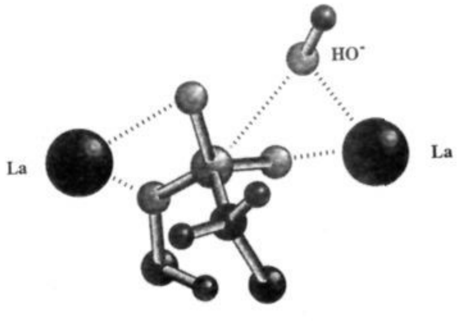
The proposed mechanism is supported by the observation that no spontaneous hydrolysis of the complex (**2**)La can be detected. Thus, hydrolysis of the phosphonate moiety requires the presence of two lanthanum cations.

No hydrolysis of **1** could be detected over a period of 6 months at pH 8 and 30 °C in the absence of added La^{3+}. We estimated the rate of hydrolysis of **1** in the following manner. In the hydrolysis of phosphate diesters, including both alkyl and aryl esters, there is a linear relationship (eq 4) between the pK_a of the leaving group and the log of the bimolecular rate constant (k_{HO^-}, M^{-1} s^{-1}, 25 °C) for hydroxide-catalyzed hydrolysis with P−O bond cleavage.[5b]

$$\log k_{HO^-} = 0.69 - 0.76\ pK_a \tag{4}$$

This relationship also holds for HO$^-$-catalyzed hydrolysis of methylphosphonate monoesters. This can be appreciated by comparison of experimental[12] (30 °C) and computed (by eq 4) values of K_{HO^-} for hydrolysis of phosphonate esters: phenyl methylphosphonate, 1.2×10^{-7} M^{-1} s^{-1} *vs* 1.8×10^{-7} M^{-1} s^{-1}; *p*-nitrophenyl methylphosphonate, 5.0×10^{-5} M^{-1} s^{-1} *vs* 1.8×10^{-5} M^{-1} s^{-1}. The pK_a (alcoholic proton) value of (8-hydroxy-2-quinolyl)methanol is safely assessed to be 1 order less than that of benzyl alcohol (pK_a = 15.48)[13] with consideration of the difference in electronic effect between phenyl and 8-hydroxyquinoline. According to eq 4, the bimolecular rate constant of hydroxide-catalyzed hydrolysis of **1** with P−O bond cleavage would be $\sim 5 \times 10^{-11}$ M^{-1} s^{-1}. This would correspond to a pseudo-first-order rate constant of $\sim 5 \times 10^{-17}$ s^{-1} at pH 8. At pH 8, k_q ($\approx k_1$) = 1.36×10^{-3} s^{-1}. Therefore, a rate enhancement of $\sim 10^{13}$ has been observed.

In conclusion, the complex of two La^{3+} with **1** provides the first example of double metal ion cooperativity consisting of an intramolecular attack of metal-bound hydroxide and Lewis-acid activation. The two metal ions acting in concert provide a remarkable 10^{13} rate enhancement in the hydrolysis of the monoalkyl phosphonate.



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