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Cleavage of Phosphate Esters by a Cyclodextrin Dimer Catalyst That Binds the Substrates Together with La³⁺ and Hydrogen Peroxide

Ronald Breslow* and Biliang Zhang

*Department of Chemistry, Columbia University
New York, New York 10027*

We have reported the synthesis and properties of cyclodextrin dimer **1**, whose linker can bind metal ions.[1] It uses them to catalyze the hydrolysis of esters that bind into both cavities and stretch along the linker group.[1] We[4] and others[5–7] have described the
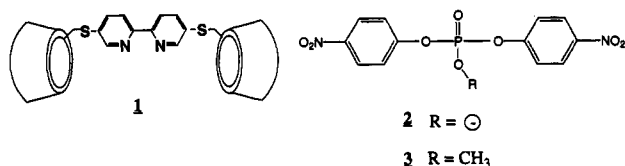
hydrolysis of phosphodiesters catalyzed by lanthanide ions, and the hydrolysis of bis(*p*-nitrophenyl) phosphate (**2**)[8,9] and of RNA[10–12] by bipyridine complexes has been reported. Rate accelerations were generally 10^3 or less. Meares has described[13] the metal ion catalyzed hydrolysis of a peptide bond in which H_2O_2 was more effective than H_2O, and such hydrolytic cleavage by hydrogen peroxide has been described recently for DNA.[14,15] Combining these approaches, Takasaki and Chin (TC) have reported[16] the remarkable catalyzed oxidative hydrolysis of bis-(*p*-nitrophenyl) phosphate (**2**) to form 2 mol of *p*-nitrophenol and inorganic phosphate using La³⁺ and H_2O_2. From the pH *vs* rate profile they suggested that there were *two* La³⁺ and two H_2O_2 in the transition state (with a related mechanism for a DNA cleavage case[17]). The acceleration at pH 7.0 exceeded 10⁸

Figure 1. Dependence of k_{obs} (s⁻¹) on [La³⁺] (M) for hydrolysis of BNPP (**2**) (0.06 mM) by hydrogen peroxide and La(III) in HEPES buffer (pH 7.0) at 25 °C, as a log/log plot.

Figure 2. Dependence of k_{obs} (s⁻¹) on [La³⁺] (M) for hydrolysis of BNPP (**2**) (0.06 mM) by dimer **1** (0.2 mM), hydrogen peroxide (48 mM), and La(III) in HEPES buffer (pH 7.0) at 25 °C, as a log/log plot.

relative to buffer alone. It seemed that the extraordinary rate effect was related to the operation of *two* complexed metal ions.

Since La³⁺ can bind to bipyridyl, we have investigated this system with our catalyst **1**. We see that there is indeed catalysis of the hydrolysis of **2** by the La³⁺ complex of **1** in the presence of H_2O_2, but with a difference in kinetic behavior from that in the absence of **1**. In particular, we find that the very large rate acceleration can be achieved with a catalyst that binds the substrate and uses only *one* La³⁺ and one H_2O_2.

We confirm the suggestion by TC that their reaction involves 2 La³⁺. As Figure 1 shows, at 25 °C and pH 7.0 (HEPES buffer) with 0.06 mM **2** and 48 mM H_2O_2 the log k *vs* log [La³⁺] is a good straight line with a slope of 2.2, from 10^{-4} M to $10^{-2.4}$ M La³⁺, where some kinetic saturation may be appearing. At 1.0 mM La³⁺ the 48 mM H_2O_2 provides an additional rate factor of 5300 relative to water. The results are essentially the same in the presence of 0.2 mM 2,2′-bipyridyl.

However, under the same conditions in the presence of 0.2 mM **1** the slope of such a log/log plot (Figure 2) is 1.03. This cannot reflect saturation binding of one La³⁺ to **1**, with only a second La³⁺ detected kinetically,[18] since (1) the linear plot extends well below the 1:1 La³⁺/**1** ratio, and (2) binding of La³⁺ is not strong enough for such saturation at low concentrations (*vide*

(1) Breslow, R.; Zhang, B. *J. Am. Chem. Soc.* **1992**, *114*, 5882–5883. We described the synthesis and the mass spectrum of **1**; the NMR spectra (¹H and ¹³C) are also definitive. For instance, ¹H NMR (400 MHz, DMSO-*d₆*): δ 3.10–4.20 (m, H₂, H₃, H₄, H₅, and H₆ on cyclodextrin), 4.50 (m, 12H, primary hydroxyl), 4.82 (m, 14H, anomeric protons), 5.75 (m, 28H, secondary hydroxyl), 7.93 (dd, *J* = 8.4 and 2.4 Hz, 2H, pyridine), 8.23 (d, *J* = 8.4 Hz, 2H, pyridine) and 8.58 (d, *J* = 2.0 Hz, 2H, pyridine). We saw the hydrolysis of a *p*-nitrophenyl ester of adamantanepropiolic acid catalyzed by the Cu²⁺ complex of **1** with a rate acceleration of 220000 fold. In more recent work we see that the Zn²⁺ complex of **1** catalyzes the hydrolysis of that ester in the presence of pyridine-2-carboxaldoxime with a rate acceleration of 1.7 × 10⁶ over the background rate at pH 7.0, apparently using the catalysis by metal complexes of this oxime that we first reported many years ago.[2,3]
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(15) Schnaith, L. M. T.; Hanson, R. S.; Que, L., Jr. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 569–73.
(16) Takasaki, B. K.; Chin, J. *J. Am. Chem. Soc.* **1993**, *115*, 9337–9338.
(17) Cf. also: Takasaki, B. K.; Chin, J. *J. Am. Chem. Soc.* **1994**, *116*, 1121–1122.

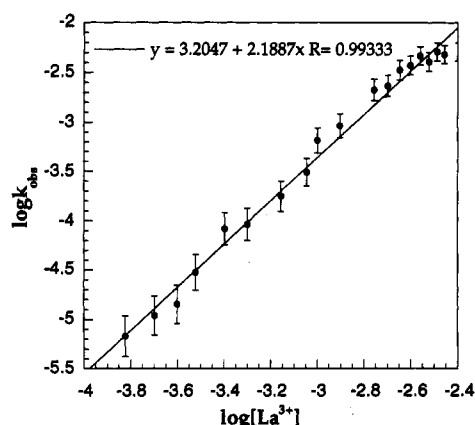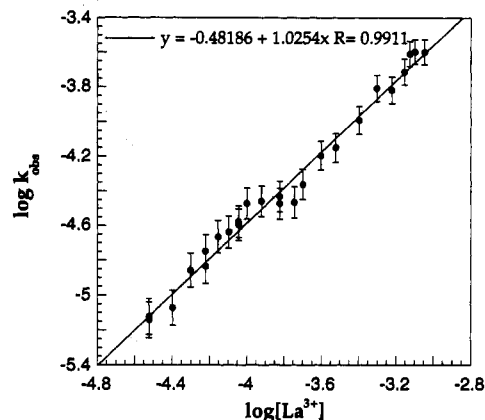(18) The saturation kinetic data with substrate **3** show that binding of La³⁺ to **1** is not complete below [La³⁺] of 1 mM, and if it were complete a second free La³⁺ would not be available when [La³⁺] < 0.2 mM, the concentration of **1**. However, these arguments do not exclude the possibility that La³⁺ is strongly bound to the *complex* of **1** and **2** (but not **1** and **3**). Additional evidence for our mechanism is our finding that the reaction with **1** and **2** has a kinetic order of 1.13 ± 0.06 in H_2O_2, while the reaction without **1** has a kinetic order of 1.75 ± 0.18 in H_2O_2.

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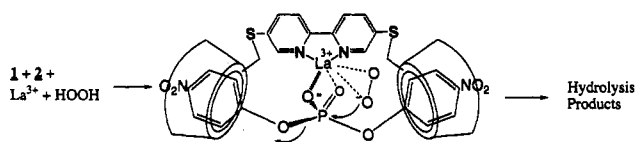
Scheme 1

infra). Instead, we have a mechanism that involves only one La^{3+} and one H_2O_2 in the transition state (we see an H_2O_2 kinetic order of 1.13 ± 0.06) , presumably that of Scheme 1. Under these conditions, with 1.0 mM La^{3+}, the 48 mM H_2O_2 provides an additional rate factor of 400 relative to water.

Consistent with the difference in kinetic order, when $[La^{3+}]$ < 0.5 mM the process with dimer 1 present is faster than without it, the rate advantage increasing as $[La^{3+}]$ is lowered (Table 1.). With $[La^{3+}]$ = 0.1 mM, for example, the rate acceleration is 9.7 × 10⁴ in the absence of 1 and 300 × 10⁴ in its presence at 0.2 mM. At $[La^{3+}]$ > 1.0 mM the dimer gives no rate advantage and can become a weak inhibitor (Table 1), presumably by binding substrate, since the process second order in La^{3+} takes over. Just as TC report,[16] we see (³¹P NMR) the formation of some monoester partway through the reactions, which eventually disappears to form only inorganic phosphate.

With phosphate triester 3,[19] the situation is different. Now there is almost no observable catalysis by La^{3+} and H_2O_2 alone (Table 1), since the neutral substrate does not bind to the metal ion. The result is that the phosphate diester 2—which is ca. 10⁵ slower in simple base hydrolysis than is the triester 3—becomes 10³ faster than 2 with 1.0 mM La^{3+} and 48 mM H_2O_2. However, in the presence of dimer 1 we see catalysis of the hydrolysis of 3 (Table 1). The rate increases linearly with $[La^{3+}]$ until it levels off to a plateau at $[La^{3+}]$ > 1.0 mM, apparently representing saturation binding of La^{3+} to 1 only at this rather high La^{3+} concentration. The product in this case is methyl phosphate (³¹P

(19) Moffatt, J. G.; Khorana, H. G. *J. Am. Chem. Soc.* **1957**, *79*, 3741. CI-MS: $(M + 1)^+$ = 355. ¹H NMR as expected with CH_3 doublet from ³¹P splitting.

Table 1. Some Pseudo-First-Order Rate Constants for Hydrolysis of Phosphate Esters in pH 7.0 Aqueous Solution at 25 °C[a]

$[La^{3+}]$ (mM)	HOOH (mM)	dimer 1 (mM)	k_{obs}[b] (s⁻¹)	k_{rel} (k_{obs}/k_{uncat})
Bis(p-nitrophenyl) Phosphate (2)				
			1.1 × 10⁻¹¹ [c]	1
0.1	48		(1.07 ± 0.45) × 10⁻⁶	9.73 × 10⁴
0.1	48	0.2	(3.37 ± 0.24) × 10⁻⁵	3.06 × 10⁶
1.0	48		(9.22 ± 0.15) × 10⁻⁴	8.38 × 10⁷
1.0	48	0.2	(4.50 ± 0.36) × 10⁻⁴	4.09 × 10⁷
2.0	48		(3.35 ± 0.12) × 10⁻³	3.05 × 10⁸
2.0	48	0.2	(1.76 ± 0.45) × 10⁻³	1.60 × 10⁸
Methyl Bis(p-nitrophenyl) Phosphate (3)				
			(0.49 ± 0.18) × 10⁻⁶	1.0
1.0			(0.53 ± 0.11) × 10⁻⁶	1.1
1.0	48		(1.10 ± 0.52) × 10⁻⁶	2.2
1.0		0.2	(0.65 ± 0.19) × 10⁻⁶	1.3
1.0	48	0.2	(1.88 ± 0.22) × 10⁻⁴	384.0

[a] Reactions were carried out in 50 mM HEPES buffer (pH 7.0 ± 0.1) and 0.06 mM bis(p-nitrophenyl) phosphate (2) at 25.0 ± 0.2 °C, monitored by UV absorbance at 400 nm. [b] k_{obs} values were calculated by a computer program ("kore") for fast reactions, product ≥95%, the correlation coefficient ≥0.9999. For slow reactions k_{obs} values were obtained by calculating the initial rate. [c] Reference 17 and unpublished confirmation of the reported rate by Dr. W. Chapman in this laboratory.

NMR, quartet at δ 1.20) along with 2 mol of p-nitrophenol. From the data in Table 1, the H_2O_2 is adding a factor of 300 over water in this case.

It is clear that the remarkable catalytic acceleration is that for the hydrolysis of anionic substrate 2 by the La^{3+}/H_2O_2 combination reported by TC.[16] However, at low concentrations of La^{3+} a quadrimolecular complex is formed of 1, La^{3+}, H_2O_2, and substrate 2 or 3. In this complex, molecular recognition and binding of the substrate combine with a highly effective catalytic group to produce an enzyme mimic with very interesting potential.