

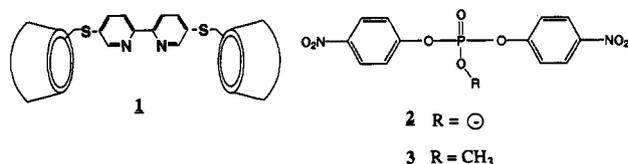
# Cleavage of Phosphate Esters by a Cyclodextrin Dimer Catalyst That Binds the Substrates Together with $\text{La}^{3+}$ and Hydrogen Peroxide

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We have reported the synthesis and properties of cyclodextrin dimer **1**, whose linker can bind metal ions.<sup>1</sup> It uses them to catalyze the hydrolysis of esters that bind into both cavities and stretch along the linker group.<sup>1</sup> We<sup>4</sup> and others<sup>5–7</sup> have described the



hydrolysis of phosphodiester catalyzed by lanthanide ions, and the hydrolysis of bis(*p*-nitrophenyl) phosphate (**2**)<sup>8,9</sup> and of RNA<sup>10–12</sup> by bipyridine complexes has been reported. Rate accelerations were generally  $10^3$  or less. Meares has described<sup>13</sup> the metal ion catalyzed hydrolysis of a peptide bond in which  $\text{H}_2\text{O}_2$  was more effective than  $\text{H}_2\text{O}$ , and such hydrolytic cleavage by hydrogen peroxide has been described recently for DNA.<sup>14,15</sup> Combining these approaches, Takasaki and Chin (TC) have reported<sup>16</sup> the remarkable catalyzed oxidative hydrolysis of bis(*p*-nitrophenyl) phosphate (**2**) to form 2 mol of *p*-nitrophenol and inorganic phosphate using  $\text{La}^{3+}$  and  $\text{H}_2\text{O}_2$ . From the pH *vs* rate profile they suggested that there were *two*  $\text{La}^{3+}$  and two  $\text{H}_2\text{O}_2$  in the transition state (with a related mechanism for a DNA cleavage case<sup>17</sup>). The acceleration at pH 7.0 exceeded  $10^8$

(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 (<sup>1</sup>H and <sup>13</sup>C) are also definitive. For instance, <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>):  $\delta$  3.10–4.20 (m, H<sub>2</sub>, H<sub>3</sub>, H<sub>4</sub>, H<sub>5</sub>, and H<sub>6</sub> 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 adamantanepropionic acid catalyzed by the  $\text{Cu}^{2+}$  complex of **1** with a rate acceleration of 220000 fold. In more recent work we see that the  $\text{Zn}^{2+}$  complex of **1** catalyzes the hydrolysis of that ester in the presence of pyridine-2-carboxaldoxime with a rate acceleration of  $1.7 \times 10^6$  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.<sup>2,3</sup>

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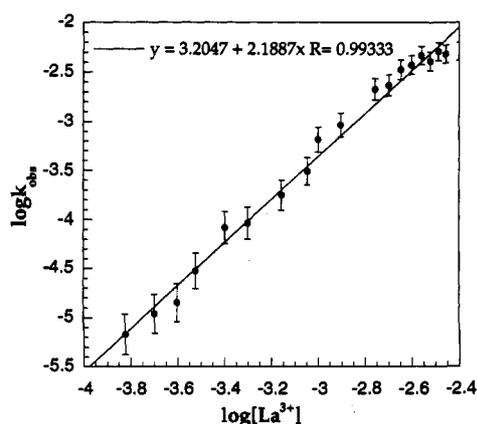
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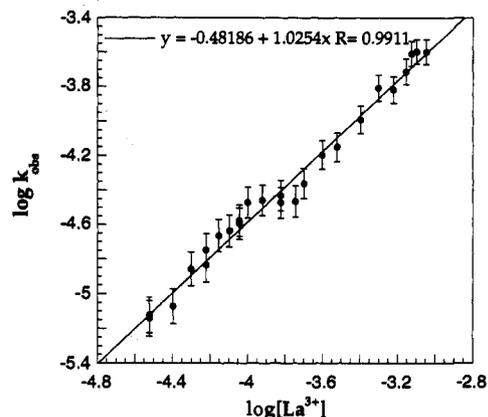
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**Figure 1.** Dependence of  $k_{\text{obs}}$  ( $\text{s}^{-1}$ ) on  $[\text{La}^{3+}]$  (M) for hydrolysis of BNPP (**2**) (0.06 mM) by hydrogen peroxide and La(III) in HEPES buffer (pH 7.0) at 25 °C, as a log/log plot.



**Figure 2.** Dependence of  $k_{\text{obs}}$  ( $\text{s}^{-1}$ ) on  $[\text{La}^{3+}]$  (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.

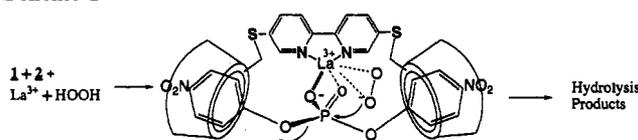
Since  $\text{La}^{3+}$  can bind to bipyridyl, we have investigated this system with our catalyst **1**. We see that there is indeed catalysis of the hydrolysis of **2** by the  $\text{La}^{3+}$  complex of **1** in the presence of  $\text{H}_2\text{O}_2$ , but with a difference in kinetic behavior 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*  $\text{La}^{3+}$  and one  $\text{H}_2\text{O}_2$ .

We confirm the suggestion by TC that their reaction involves 2  $\text{La}^{3+}$ . As Figure 1 shows, at 25 °C and pH 7.0 (HEPES buffer) with 0.06 mM **2** and 48 mM  $\text{H}_2\text{O}_2$  the  $\log k$  *vs*  $\log [\text{La}^{3+}]$  is a good straight line with a slope of 2.2, from  $10^{-4}$  M to  $10^{-2.4}$  M  $\text{La}^{3+}$ , where some kinetic saturation may be appearing. At 1.0 mM  $\text{La}^{3+}$  the 48 mM  $\text{H}_2\text{O}_2$  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  $\text{La}^{3+}$  to **1**, with only a second  $\text{La}^{3+}$  detected kinetically,<sup>18</sup> since (1) the linear plot extends well below the 1:1  $\text{La}^{3+}$ /**1** ratio, and (2) binding of  $\text{La}^{3+}$  is not strong enough for such saturation at low concentrations (*vide*

(18) The saturation kinetic data with substrate **3** show that binding of  $\text{La}^{3+}$  to **1** is not complete below  $[\text{La}^{3+}]$  of 1 mM, and if it were complete a second free  $\text{La}^{3+}$  would not be available when  $[\text{La}^{3+}] < 0.2$  mM, the concentration of **1**. However, these arguments do not exclude the possibility that  $\text{La}^{3+}$  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  $1.13 \pm 0.06$  in  $\text{H}_2\text{O}_2$ , while the reaction without **1** has a kinetic order of  $1.75 \pm 0.18$  in  $\text{H}_2\text{O}_2$ .

## Scheme 1



*infra*). Instead, we have a mechanism that involves only one  $\text{La}^{3+}$  and one  $\text{H}_2\text{O}_2$  in the transition state (we see an  $\text{H}_2\text{O}_2$  kinetic order of  $1.13 \pm 0.06$ ), presumably that of Scheme 1. Under these conditions, with 1.0 mM  $\text{La}^{3+}$ , the 48 mM  $\text{H}_2\text{O}_2$  provides an additional rate factor of 400 relative to water.

Consistent with the difference in kinetic order, when  $[\text{La}^{3+}] < 0.5$  mM the process with dimer 1 present is faster than without it, the rate advantage increasing as  $[\text{La}^{3+}]$  is lowered (Table 1). With  $[\text{La}^{3+}] = 0.1$  mM, for example, the rate acceleration is  $9.7 \times 10^4$  in the absence of 1 and  $300 \times 10^4$  in its presence at 0.2 mM. At  $[\text{La}^{3+}] > 1.0$  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  $\text{La}^{3+}$  takes over. Just as TC report,<sup>16</sup> we see ( $^{31}\text{P}$  NMR) the formation of some monoester partway through the reactions, which eventually disappears to form only inorganic phosphate.

With phosphate triester 3,<sup>19</sup> the situation is different. Now there is almost no observable catalysis by  $\text{La}^{3+}$  and  $\text{H}_2\text{O}_2$  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.  $10^5$  slower in simple base hydrolysis than is the triester 3—becomes  $10^3$  faster than 2 with 1.0 mM  $\text{La}^{3+}$  and 48 mM  $\text{H}_2\text{O}_2$ . However, in the presence of dimer 1 we see catalysis of the hydrolysis of 3 (Table 1). The rate increases linearly with  $[\text{La}^{3+}]$  until it levels off to a plateau at  $[\text{La}^{3+}] > 1.0$  mM, apparently representing saturation binding of  $\text{La}^{3+}$  to 1 only at this rather high  $\text{La}^{3+}$  concentration. The product in this case is methyl phosphate ( $^{31}\text{P}$

**Table 1.** Some Pseudo-First-Order Rate Constants for Hydrolysis of Phosphate Esters in pH 7.0 Aqueous Solution at 25 °C<sup>a</sup>

$[\text{La}^{3+}]$ (mM)	HOOH (mM)	dimer 1 (mM)	$k_{\text{obs}}^b$ (s <sup>-1</sup> )	$k_{\text{rel}}$ ( $k_{\text{obs}}/k_{\text{uncat}}$ )
Bis( <i>p</i> -nitrophenyl) Phosphate (2)				
			$1.1 \times 10^{-11}$ <sup>c</sup>	1
0.1	48		$(1.07 \pm 0.45) \times 10^{-6}$	$9.73 \times 10^4$
0.1	48	0.2	$(3.37 \pm 0.24) \times 10^{-5}$	$3.06 \times 10^6$
1.0	48		$(9.22 \pm 0.15) \times 10^{-4}$	$8.38 \times 10^7$
1.0	48	0.2	$(4.50 \pm 0.36) \times 10^{-4}$	$4.09 \times 10^7$
2.0	48		$(3.35 \pm 0.12) \times 10^{-3}$	$3.05 \times 10^8$
2.0	48	0.2	$(1.76 \pm 0.45) \times 10^{-3}$	$1.60 \times 10^8$
Methyl Bis( <i>p</i> -nitrophenyl) Phosphate (3)				
			$(0.49 \pm 0.18) \times 10^{-6}$	1.0
1.0			$(0.53 \pm 0.11) \times 10^{-6}$	1.1
1.0	48		$(1.10 \pm 0.52) \times 10^{-6}$	2.2
1.0		0.2	$(0.65 \pm 0.19) \times 10^{-6}$	1.3
1.0	48	0.2	$(1.88 \pm 0.22) \times 10^{-4}$	384.0

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

NMR, quartet at  $\delta$  1.20) along with 2 mol of *p*-nitrophenol. From the data in Table 1, the  $\text{H}_2\text{O}_2$  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  $\text{La}^{3+}/\text{H}_2\text{O}_2$  combination reported by TC.<sup>16</sup> However, at low concentrations of  $\text{La}^{3+}$  a quadrimolecular complex is formed of 1,  $\text{La}^{3+}$ ,  $\text{H}_2\text{O}_2$ , 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.

**Acknowledgment.** Support of this work by the National Institutes of Health and the Office of Naval Research are gratefully acknowledged.

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