

# Dinuclear lanthanum complex catalyzes the hydrolysis of a phosphate diester with unprecedented speed

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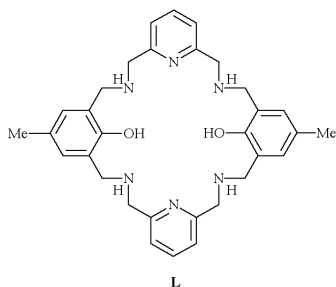
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Received (in Bloomington, IN, USA) 23rd October 1998, Accepted 12th July 1999

The dihydroxo dilanthanum macrocyclic complex hydrolyzes bis(*p*-nitrophenyl) hydrogen phosphate (BNP) with a remarkably high rate constant and a unique third-order dependence on the concentration of the dinuclear complex.

Restriction endonucleases are enzymes that hydrolyze the phosphate diester bonds of DNA with great specificity and high turnover numbers.<sup>1</sup> A small molecule that could recognize a specific DNA sequence and catalyze the rapid hydrolysis of the phosphate diester bond would be an invaluable research tool. Multinuclear metal complexes have been known to hydrolyze phosphate diesters<sup>2</sup> and are models for a number of phosphodiesterases that contain multinuclear active sites.<sup>3</sup> This work reports a defined dinuclear lanthanum(III) complex that can hydrolyze a phosphate diester (BNP) with unprecedented speed. This complex also has the unique feature that the hydrolysis of BNP is third-order with respect to the concentration of the dinuclear La<sup>3+</sup> complex, [LLa<sub>2</sub>].

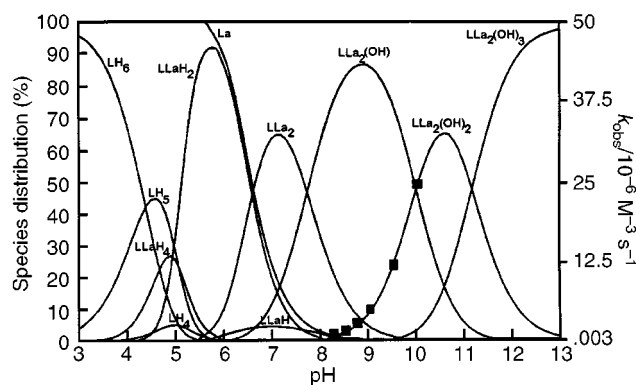
The ligand L was synthesized according to literature procedures.<sup>4</sup> Potentiometric experiments were performed by



means of a well-defined method<sup>5</sup> to determine stability constants for the mono- and di-nuclear La<sup>3+</sup> complexes (Table 1). The p*K*<sub>a</sub> of a water bound to the mononuclear complex [LLa] is 11.15, while the p*K*<sub>a</sub> of a water bound to the dinuclear complex [LLa<sub>2</sub>] is 7.75. The fact that the p*K*<sub>a</sub> is lowered by 3.40

**Table 1** Stability constants for the formation of mono- and di-nuclear La<sup>3+</sup> complexes with the ligand L. *T* = 35 °C,  $\mu$  = 0.10 M KCl, 3:1 EtOH–H<sub>2</sub>O solution p*K*<sub>a</sub>s of L, under the same conditions: 12.95, 12.84, 8.24, 7.39, 5.76 and 4.38

Equilibrium	Log <i>K</i>
[LLa]/[L][La]	18.52
[LHLa]/[LH][La]	14.48
[LH <sub>2</sub> La]/[LH <sub>2</sub> ][La]	9.31
[LH <sub>3</sub> La]/[LH <sub>3</sub> ][La]	6.23
[LH <sub>4</sub> La]/[LH <sub>4</sub> ][La]	3.56
[LLa(OH)]/[H][LLa]	11.15
[LLa <sub>2</sub> ]/[LLa][La]	6.67
[LLa <sub>2</sub> (OH)]/[H][LLa <sub>2</sub> ]	7.75
[LLa <sub>2</sub> (OH) <sub>2</sub> ]/[H]/[LLa <sub>2</sub> (OH)]	10.03
[LLa <sub>2</sub> (OH) <sub>3</sub> ]/[H]/[LLa <sub>2</sub> (OH) <sub>2</sub> ]	11.17



**Fig. 1** Species distribution diagram for a system containing a 1:2 ratio L to La<sup>3+</sup>. Observed third-order rate constants are denoted by (■).

pH units implies that the water is bridging the two La<sup>3+</sup> ions. The p*K*<sub>a</sub> of the second water bound to the dinuclear complex is 10.03, so this water also forms a bridge between the two La<sup>3+</sup> ions.

In Fig. 1 the prevalence of the various dinuclear  $\mu$ -hydroxy species can be seen. In the pH range 6–8 the rate constants are easily measurable, but relatively small in magnitude. The graph clearly shows that the rate constants follow the formation of the dinuclear dihydroxo complex [LLa<sub>2</sub>(OH)<sub>2</sub>]. When [LLa<sub>2</sub>]<sub>TOT</sub> = [BNP]<sub>INIT</sub> = 0.5 mM, it would take 5.0 days for one turnover at pH 7.<sup>6</sup> It would take only 27 min for one turnover at pH 9 and a mere 6 min at pH 10. It is not clear why the dihydroxo complex is more effective than the monohydroxo complex [LLa<sub>2</sub>(OH)]. There is at least one other example in the literature of a dinuclear macrocyclic complex where the active catalyst for hydrolyzing BNP is the dihydroxo complex and not the analogous monohydroxo species.<sup>2a</sup> The reason may simply be that the second hydroxo ligand is better positioned for nucleophilic attack than the initial hydroxo complex that forms.

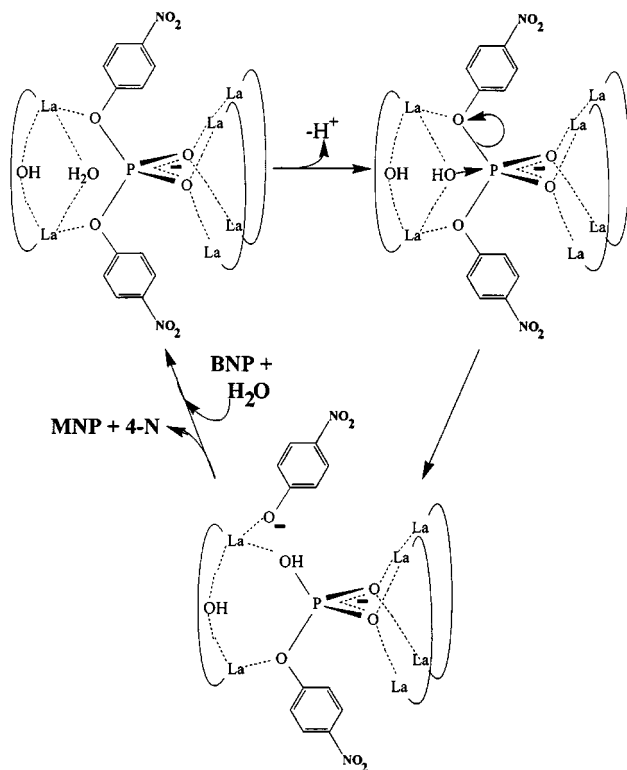
Within the concentration range studied, the reaction is first-order with respect to [BNP]<sub>INIT</sub>. However, analysis of the data in Table 2 shows that the reaction is an unusual third-order with respect to the concentration of the dinuclear species [LLa<sub>2</sub>]<sub>TOT</sub>. Consequently, the rate law is given by eqn. (1).

$$\text{Rate} = k_{\text{obs}}[\text{LLa}_2]_3[\text{TOT}][\text{BNP}]_{\text{INIT}} \quad (1)$$

A reaction may appear to have an unusual catalyst dependence if there is an unexpected equilibrium at the concentration at which the kinetic trials were measured as opposed to the

**Table 2** Dependence of the rate of BNP hydrolysis on the concentration of [LLa<sub>2</sub>]<sub>TOT</sub>

pH	[LLa <sub>2</sub> ] <sub>TOT</sub> /M	[BNP] <sub>INIT</sub> /M	Rate/10 <sup>8</sup> M s <sup>-1</sup>
8.25	0.000728	0.000531	24
8.25	0.000486	0.000539	7.3
8.25	0.000244	0.000518	0.81



**Scheme 1** Proposed mechanism for BNP hydrolysis by  $[\text{LLa}_2(\text{OH})_2]$ . For simplicity, the ligand has been drawn as a curved line and the coordinated hydroxides of the two rightmost  $[\text{LLa}_2(\text{OH})_2]$  species were not included.

concentration at which the potentiometric experiments were performed. To test this hypothesis the stability constants were measured over a large concentration range. The stability constants at various concentrations agreed to within experimental error. Thus this aggregation of three  $[\text{LLa}_2]$  molecules, required to satisfy the third-order rate law dependence, occurs only in the presence of substrate.

A proposed mechanism is shown in Scheme 1. Two dinuclear  $\text{La}^{3+}$  complexes coordinate the partially negatively charged phosphate oxygens. The third dinuclear  $\text{La}^{3+}$  complex coordinates both ester groups and attacks the phosphorus center with one of its bridging hydroxide nucleophiles. A *p*-nitrophenolate group (4-N) is displaced and the monophosphate ester (MNP) is

generated. The products are displaced and the  $[\text{LLa}_2]$  complex molecules coordinate another incoming substrate. The active catalyst  $[\text{LLa}_2(\text{OH})_2]$  is regenerated by the loss of a proton from a coordinated water. Hydrolysis is fast because there are three dinuclear  $\text{La}^{3+}$  complexes withdrawing electron density from the phosphorus center. This makes that phosphorus center extremely susceptible to nucleophilic attack from the coordinated hydroxide nucleophile.

This work describes the formation of the first defined dinuclear  $\text{La}^{3+}$  complex capable of hydrolyzing phosphate diesters. The catalysis occurs at such an unprecedented rate that it takes only 6 min for one turnover at pH 10. The rate constant *versus* pH profile clearly shows the most efficient catalyst is the dinuclear dihydroxo complex  $[\text{LLa}_2(\text{OH})_2]$ . The unique third-order dependence on  $[\text{LLa}_2]_{\text{TOT}}$  is the most probable reason for the high rate constants that are observed. The high rate constants and third-order concentration dependence holds much promise for future work of hydrolyzing DNA on a practical time scale.

## Notes and references

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- 6 A  $k_{\text{cat}}$  value of  $0.0004 \text{ s}^{-1}$  was measured at pH 7.0 by using  $[\text{LLa}_2]_{\text{TOT}} = 5.0 \text{ mM}$  and  $[\text{BNP}]_{\text{INIT}} = 0.5 \text{ mM}$ . The rate enhancement over spontaneous hydrolysis is  $1.2 \times 10^7$ . A  $k_{\text{cat}}$  value at pH 10.0, with the concentrations just described, is too fast to measure by our method. By extrapolation, it calculates to  $k_{\text{cat}} = 0.32 \text{ s}^{-1}$  which is one turnover every 3–4 s. This is a rate enhancement of  $1.2 \times 10^8$ .

Communication 9/00860H