Rapid Cleavage of RNA with a La(III) Dimer

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In nature there are many enzymes that hydrolyze phosphate esters that are activated by two or more metal ions. They include phosphomonoesterases[1] (alkaline phosphatase,[2] purple acid phosphatase,[3] inositol phosphatase[4]), phosphodiesterases (RNase H from HIV reverse transcriptase,[5] 3′−5′ exonuclease from DNA polymerase I,[6] P1 nuclease[7]), and phosphotriesterases (isolated from *Pseudomonas diminuta*[8]). Inspired by nature's enzymes, many simple dinuclear metal complexes that hydrolyze phosphate esters have been developed.[9] Currently there is considerable interest in developing catalysts that hydrolyze RNA sequence specifically.[10] One major challenge in such an endeavor is increasing the reactivity of the catalyst. Here we report La(III) ions that dimerize to form a reactive core that cleaves RNA with unprecedented reactivity.

Potentiometric titration (supporting information) of an aqueous solution of $LaCl_3$ (2 mM) shows the consumption of approximately 2.5 equiv of hydroxide at about pH 9.[11] The steepness of the titration curve suggests that a dimer or higher order aggregates are formed.[9c] Scheme 1 describes a simple dimerization reaction that requires 2.5 equiv of hydroxide. The equations for the equilibrium constant for dimerization (eq 1) and the conservation of mass (eq 2) may be solved for the concentration of the proposed dimer as a function of hydrogen ion concentration (eq 3). The dimer concentration can in turn

$$K = \frac{[La_2(OH)_5^{+}]}{[La]^2[OH]^5} \tag{1}$$

$$[La] + 2[La_2(OH)_5^{+}] = [La]_t \tag{2}$$

$$[La_2(OH)_5^{+}] = \frac{[H]^5 + 4[La]_t K K_w^{\,5} - ([H]^{10} + 8[H]^5[La]_t K K_w^{\,5})^{1/2}}{8 K K_w^{\,5}} \tag{3}$$

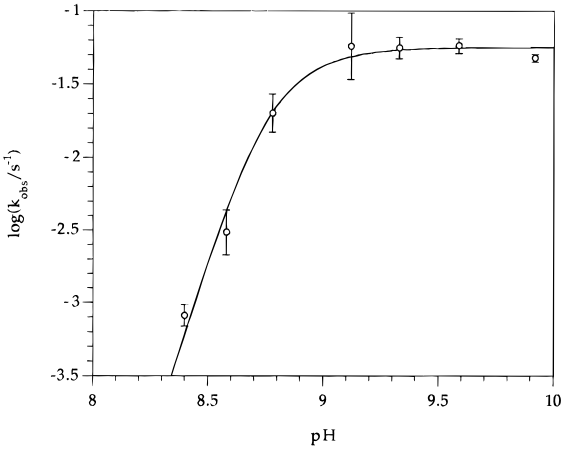

Figure 1. pH−rate profile for $La_2(OH)_5^{+}$ ($[La]_t$ = 2 mM)-promoted hydrolysis of ApA (0.025 mM) in 20 mM EPPS or CHES at 25 °C. Calculated curve derived from eq 5.

Scheme 1

$$2La(III) + 5OH^- \overset{K}{\rightleftharpoons} La_2(OH)_5^{+}$$

be related to the concentration of the added hydroxide in the potentiometric titration (eq 4). The best fit of the titration data (supporting information) according to eq 4 (where $[La_2(OH)_5^{+}]$ is given by eq 3 and $[OH]_t$ is amount of hydroxide added) gave $K = (1.4 \pm 0.1) \times 10^{28}$ M^{-6}.

$$[OH]_t = 5[La_2(OH)_5^{+}] \tag{4}$$

Hydrolysis of ApA (0.025 mM) in aqueous solutions of $LaCl_3$ (1−2 mM) at pH 8.4−9.9 (20 mM EPPS or CHES buffer) and 25 °C was monitored by HPLC. Low concentrations of $LaCl_3$ were used to prevent gel formation. At appropriate time intervals, reactions were quenched by pipetting aliquots of the reaction mixture into equal volumes of an ammonium phosphate buffer (0.2 M, pH 5.5).[12] The HPLC chromatograms (supporting information) reveal that hydrolysis of ApA is clean and rapid and goes to completion producing only adenosine, 3′-adenosine phosphate, and 2′-adenosine phosphate.

In aqueous solutions of $LaCl_3$, the rates of cleavage of ApA increase sharply (slope of ∼5) with increase in solution pH but level off at pH above the midpoint for the potentiometric titration of $LaCl_3$ (Figure 1). The pH-rate profile was fit according to eq 5, where k is the second-order rate constant for $[La_2(OH)_5^{+}]$ promoted cleavage of ApA. The best fit of the pH−rate profile

$$k_{obs} = k[La_2(OH)_5^{+}] \tag{5}$$

gave $K = (2.8 \pm 0.6) \times 10^{28}$ M^{-6} and $k = 57 \pm 6$ M^{-1} s^{-1}. Thus the K value obtained from the pH−rate profile is in excellent agreement with that obtained from the potentiometric titration, indicating that the active species for cleaving ApA is $[La_2(OH)_5^{+}]$ or its kinetic equivalent. Also consistent with the proposed dimer as the kinetically active species are the steep

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(12) Precipitates were centrifuged out (132 000 rpm, 5 min) prior to HPLC analysis. Products were analyzed with a Hewlett Packard 1090 HPLC using a 100 × 2.1 mM C-18 column running 0.5 mL/min of 0.2 M $NH_4H_2PO_4$ buffer (pH 5.5) for the first 5 min followed by a linear gradient to 50% of a 60/40 methanol/water solution over the next 10 min. All of the rate constants were obtained by first-order kinetics methods (first three half-lives) except for the slowest rate in Figure 1, which was obtained by initial rate methods. The data points in Figures 1 and 2 are averages of at least three runs, and the error bars represent (±) three standard deviations.

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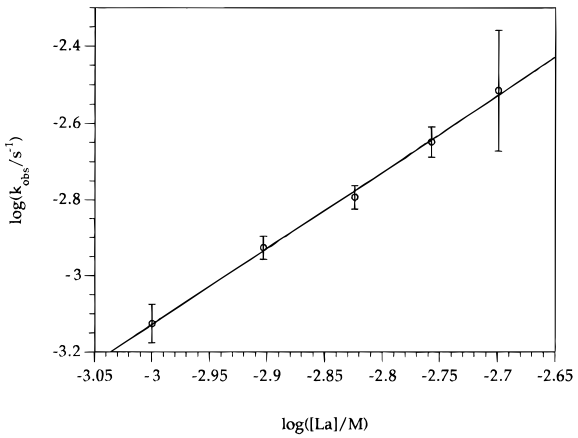


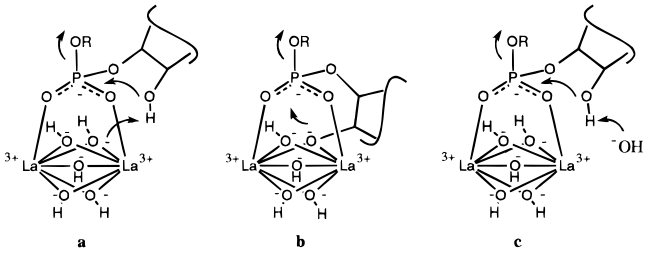
Figure 2. Dependence of the logarithm of the pseudo-first-order rate constant for the cleavage of ApA on the logarithm of $[La]_t$ at pH 8.58 (20 mM EPPS) and 25 °C (slope = 2.00 ± 0.05).

slope in the pH−rate profile (fifth order in hydroxide) and a second-order dependence upon lanthanum concentration (Figure 2).

Mononuclear Ln(III) ions[13] and their complexes[14] have been shown to cleave RNA efficiently. Interestingly, $La_2(OH)_5^+$ is about 4 orders of magnitude more reactive than mononuclear Ln(III) ions or complexes for hydrolyzing ApA. The half-life of ApA at the plateau of the pH−rate profile (Figure 1) is only about 13 s. Although 2′,3′-cAMP does not accumulate to an observable extent during the reaction, it is likely to be an intermediate since dApdA is not cleaved by the La(III) dimer. Furthermore, product analysis shows that the same ratio of 2′-AMP to 3′-AMP is produced from the $La_2(OH)_5^+$-promoted cleavage of ApA and 2′,3′-cAMP. The latter reaction is too fast to determine a rate constant by our HPLC methods.

The exact structure of $La_2(OH)_5^+$ is unclear. We propose that $La_2(OH)_5^+$ may consist of two La(III) ions bridged by five hydroxides similar to the way that two peroxides have been shown to bridge two lanthanides.[15,9b] Phosphates may then bridge the dinuclear complex similar to the way that carboxylates bridge dinuclear lanthanides.[16] Potentiometric titration shows only a single inflection (at pH ∼9), indicating that the coordination of hydroxides to lanthanum is cooperative. Multiple inflections may be expected if some of the hydroxides in $La_2(OH)_5^+$ are bridging while others are not.[17] Two mechanisms that are consistent with all our data are shown in Scheme 2a,b. One of the bridging hydroxides could act as an intramolecular general base catalyst (Scheme 2a) for the hydrolysis reaction. Alternatively, the 2′-alkoxide could replace one of the bridging hydroxides (Scheme 2b) and the metal alkoxide could act as the nucleophile. The observed pH−rate profile is inconsistent with the mechanism involving uncoordinated 2′-alkoxide as the nucleophile (Scheme 2c). Above pH 9, the slope of the pH−rate profile for such a mechanism (Scheme 2c) should be unity.

Scheme 2



In conclusion, potentiometric titration reveals that, above pH 9, La(III) ions dimerize to form $La_2(OH)_5^+$. The rate of La(III)-promoted hydrolysis of ApA increases sharply with increase in pH but levels off above pH 9, indicating that $La_2(OH)_5^+$ is the active species. Below pH 9, the rate of hydrolysis of ApA is second order in [La] and fifth order in [OH]. Interestingly, $La_2(OH)_5^+$ is about 10^4 times more reactive than previously reported mononuclear Ln(III) ions or complexes for hydrolyzing ApA. The half-life for $La_2(OH)_5^+$ (1 mM)-promoted hydrolysis of ApA is about 13 s at 25 °C. To our knowledge this represents the fastest non-enzymic hydrolysis of ApA reported to date. We are working to develop organic ligands that bind tightly to this dinuclear core.

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Supporting Information Available: Potentiometric titration of $LaCl_3$ and HPLC chromatograms for $La_2(OH)_5^+$-promoted hydrolysis of ApA (3 pages). See any current masthead page for ordering and Internet access instructions.

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(17) However, the kinetically reactive species of the catalyst may not be the thermodynamically most stable.