

## Metal Ion Promoted Attack of an Alcohol on a Phosphate Diester: Modelling the Role of Metal Ions in RNA Self-splicing Reactions

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A lanthanide(III) complex of a macrocycle containing hydroxyethyl groups promotes nucleophilic attack of an hydroxyethyl group on a phosphate diester.

The role of metal ions in facilitating the self-splicing reactions of catalytic RNAs is not well understood. For the Tetrahymena ribozyme it has been proposed that a metal ion binds to the 3'-bridging oxygen of the phosphate diester to stabilize the negative charge on the leaving group.<sup>1</sup> In addition, a second metal ion may bind to and activate the incoming 2'-hydroxy nucleophile of guanosine. Binding of a second metal ion is proposed based on the similarity of ribozyme catalysed reactions to those catalysed by protein phosphotransferases.<sup>2</sup> Certain protein phosphotransferases use two metal ions, one to activate the nucleophile and one to activate the leaving group for departure. For example there are two zinc(II) ions in alkaline phosphatase that may work in concert to activate the attacking serine residue as well as to assist leaving group departure.<sup>3</sup> Recent work modeling alkaline phosphatase has led to the finding that Zn(II) complexes promote nucleophilic attack of alcohol groups on carboxyester<sup>4,5</sup> and phosphate triesters.<sup>6</sup>

Metal ion promoted hydrolysis of phosphate diesters generally proceeds by coordination of the phosphate ester to the metal ion followed by intramolecular attack of a water or an hydroxide ligand.<sup>7,8</sup> In comparison, there are few examples of metal ion facilitated nucleophilic attack with ligands other than water or hydroxide. Other metal ion bound nucleophiles that attack phosphate diesters include peroxide ligands<sup>9,10</sup> and amido ligands.<sup>11</sup> Despite the prevalence of alcohol and alkoxide ligands as nucleophiles in RNA chemistry and in metalloenzymes there are few well-documented examples of metal ion promoted attack of these ligands on phosphate diesters. Here we report metal ion mediated attack of an alcohol group on a phosphate diester. Metal ion facilitated attack of alcohols on phosphate diesters has been proposed;<sup>12</sup> however this proposal was not supported by product analysis.

Lanthanide(III) complexes of tetraazamacrocycles containing hydroxyalkyl groups have the hydroxyalkyl groups bound to the lanthanide ion both in solution and in the solid state. The crystal structure of [Eu(THP)(H<sub>2</sub>O)](CF<sub>3</sub>SO<sub>3</sub>)<sub>3</sub> [THP = 1,4,7,10-tetra-

kis(2-hydroxypropyl)-1,4,7,10-tetraazacyclododecane] has an octadentate macrocyclic ligand with all four hydroxypropyl groups bound to Eu<sup>III</sup> and a single bound water molecule completing the coordination sphere to give a nine-coordinate Eu<sup>III</sup>.<sup>13</sup> In aqueous solution, the THP and THED ligands [THED = 1,4,7,10-tetrakis(2-hydroxyethyl)-1,4,7,10-tetraazacyclododecane, Scheme 1] remain strongly bound to lanthanide ions and the complexes are highly inert to metal ion dissociation.<sup>13,14</sup> All lanthanide(III) THED and THP complexes have an acidic group with a relatively low p*K*<sub>a</sub>. The acidic group is assigned to either a lanthanide ion bound water molecule or alternately, a lanthanide ion bound hydroxyalkyl group. As the lanthanide series is traversed from La<sup>III</sup> to Lu<sup>III</sup> the p*K*<sub>a</sub> of this group becomes lower, consistent with the increasing Lewis acidity of the heavier lanthanide ions.<sup>15</sup>

Treatment of bis(4-nitrophenyl)phosphate (BNPP) with [Eu(THED)]<sup>3+</sup> at either 37 or 25 °C, pH 7.4, leads to the rapid production of 4-nitrophenolate. The reaction is first order in BNPP over four half-lives with 1 equiv. of 4-nitrophenolate produced over this period. Pseudo-first-order rate constants for the production of 4-nitrophenolate by 1.00 mmol dm<sup>-3</sup> [Eu(THED)]<sup>3+</sup> at pH 7.4 are 2.0 × 10<sup>-4</sup> and 9.0 × 10<sup>-5</sup> s<sup>-1</sup> at 37 and 25 °C, respectively. The rate constant for the reaction at 37 °C is approximately two-fold lower in the presence of 0.10 mol dm<sup>-3</sup> NaCl. The addition of 1.0 mmol dm<sup>-3</sup> THED did not increase the production of 4-nitrophenolate species over that of the control. The rate constant for the hydrolysis of BNPP in the absence of catalyst has been estimated as 1.3 × 10<sup>-11</sup> s<sup>-1</sup> (25 °C).<sup>16</sup> Thus [Eu(THED)]<sup>3+</sup> at 1.00 mmol dm<sup>-3</sup> concentrations gives a rate enhancement of nearly 10<sup>7</sup>.

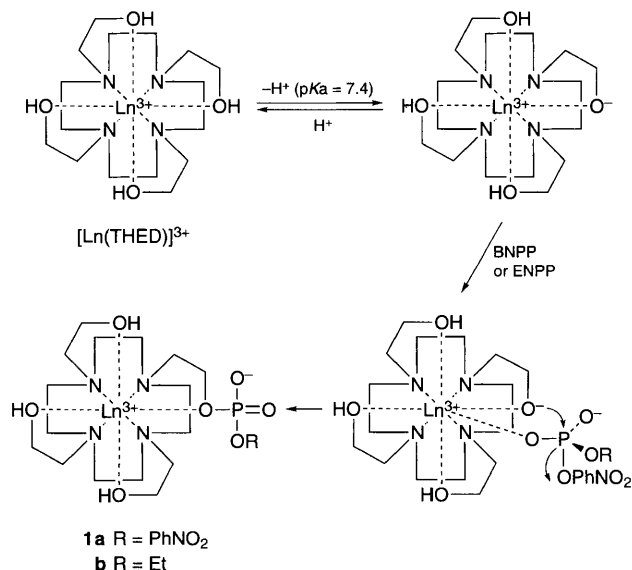
Comparison of rate constants for production of nitrophenol from BNPP by other metal ion complexes suggests that the [Eu(THED)]<sup>3+</sup> complex is among the most efficient reported to date. At 25 °C, pH 7.4, the [Eu(THED)]<sup>3+</sup> is as active as the [Co(cyclen)]<sup>3+</sup> complex in promoting the production of 4-nitrophenolate ion from BNPP.<sup>16</sup> The pseudo-first-order rate constant for the formation of 4-nitrophenol ion from BNPP by [La(THED)]<sup>3+</sup> (2.7 × 10<sup>-5</sup> s<sup>-1</sup>) is ten-fold less than that of the Eu<sup>III</sup> complex, consistent with the lower Lewis acidity of the La<sup>III</sup> complex.<sup>15</sup>

A first-order dependence on the [Eu(THED)]<sup>3+</sup> complex concentration is observed in the concentration range 0.1–1.0 mmol dm<sup>-3</sup> at 37 °C, consistent with the rate law in eqn. (1). Second-order rate constants plotted against pH (Fig. 1) give a sigmoidal pH-rate profile. Fitting of the data to eqn. (2) gives values of 4.2 × 10<sup>-8</sup> and 0.19 dm<sup>3</sup> mol<sup>-1</sup> s<sup>-1</sup> for *K*<sub>a</sub> and *k*<sub>2</sub>, respectively. The p*K*<sub>a</sub> of 7.4 obtained from the kinetic data matches closely to the value measured by potentiometric titration (7.5), suggesting that the active form of the Eu<sup>III</sup> catalyst has a bound hydroxide or alkoxide group.<sup>15</sup> Under conditions of excess BNPP where catalytic turnover might be observed, the reaction is stoichiometric. This stands in contrast to the cyclization of a phosphate diester where the reaction is catalytic in [Eu(THED)]<sup>3+</sup>.<sup>17</sup>

$$\text{Rate} = k[\text{BNPP}][\text{Eu}(\text{THED})^{3+}] \quad (1)$$

$$k = K_a k_2 / (K_a + [\text{H}^+]) \quad (2)$$

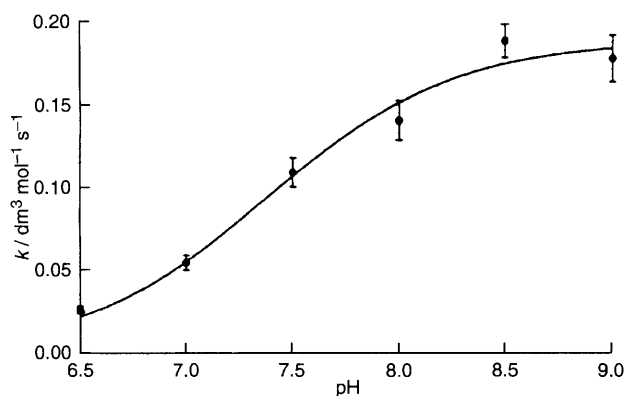
Analysis of products by <sup>31</sup>P NMR spectroscopy and by HPLC support the formation of a new phosphate diester resulting from



displacement of 4-nitrophenolate by a nucleophile other than water or hydroxide. None of the hydrolysis product, nitrophenylphosphate (NPP), is detected by use of  $^{31}\text{P}$  NMR nor by use of HPLC. HPLC analysis of the reaction confirmed the production of 4-nitrophenol as a product. Over the course of the reaction of BNPP with  $[\text{Eu}(\text{THED})]^{3+}$  and  $[\text{La}(\text{THED})]^{3+}$  new  $^{31}\text{P}$  resonances at  $\delta$  5.6 $\dagger$  and 4.8, respectively, increase in intensity. The  $[\text{La}(\text{THED})]^{3+}$  complex containing the bound phosphate diester is readily isolated in analytically pure form as  $[\mathbf{1a}](\text{CF}_3\text{SO}_3)_2$  (Scheme 1) by treatment of the complex with 2 equiv. of sodium hydroxide and the free acid form of BNPP in ethanol.

Similar products are obtained with other phosphate diesters. For example, treatment of  $[\text{La}(\text{THED})]^{3+}$  with ethyl(4-nitrophenyl)phosphate produces 4-nitrophenolate and a new  $^{31}\text{P}$  resonance at  $\delta$  1.1 suggesting formation of complex  $\mathbf{1b}$ .  $^{31}\text{P}$  NMR experiments carried out after removal of the lanthanum ion with oxalate also support the formation of  $\mathbf{1b}$ . The  $^{31}\text{P}$  resonance in the NMR spectrum of the free macrocycle of  $\mathbf{1b}$  is a pseudo-quintet due to  $^1\text{H}$ - $^{31}\text{P}$  coupling ( $J_{\text{P-H}} = 6.5$  Hz). Selective  $^1\text{H}$ - $^{31}\text{P}$  decoupling experiments demonstrate that the  $\text{CH}_2\text{O}$  of an hydroxyethyl group of the macrocyclic ligand as well as the methylene protons of the ethyl group of the phosphate diester are coupled to the  $^{31}\text{P}$  resonance.

All data are consistent with metal ion facilitated intermolecular transesterification of the phosphate diesters (Scheme 1). The stoichiometric nature of the reaction is attributed to a loss of catalytic activity upon formation of  $\mathbf{1}$ . Product analysis and the pH-rate profile suggest that a bound alkoxide group of the macrocyclic complex attacks the phosphate diester to displace 4-nitrophenolate. The increased acidity of hydroxyethyl groups when bound to metal ions is well-documented. $^{4,\ddagger}$  Alternately as observed recently for a  $\text{Zn}^{\text{II}}$  hydroxyalkyl complex, $^5$  we cannot rule out formation of a metal hydroxide complex at alkaline pH



**Fig. 1** The pH-rate profile at 37 °C [ $\mu = 0.1$  mol dm $^{-3}$  (NaCl)] for the production of 4-nitrophenolate from BNPP in the presence of 1.00 mmol dm $^{-3}$   $[\text{Eu}(\text{THED})]^{3+}$ . Data is fit according to Eqn. (2).

followed by deprotonation of the hydroxyethyl group by the hydroxide ligand.

This is, to our knowledge, the first documented example of metal ion facilitated attack of an alcohol group on a phosphate diester and demonstrates that metal ions may promote these reactions that may be of importance in ribozyme chemistry.

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### Footnote

$\dagger$   $^{31}\text{P}$  NMR chemical shifts are reported vs. 85% phosphoric acid as a reference. Chemical shifts downfield of the reference are positive.

$\ddagger$  Since this paper was submitted, two examples have been reported (refs. 18, 19).

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