

## Synergetic Catalysis by Two Non-lanthanide Metal Ions for Hydrolysis of Diribonucleotides

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Adenylyl(3'-5')adenosine and uridylyl(3'-5')uridine are efficiently hydrolysed at pH 7 by bimetallic cooperation of zinc(II) with tin(IV), indium(III), iron(III) or aluminium(III).

Non-enzymatic hydrolysis of RNA has attracted much interest, and a variety of catalysts for this process have been reported.<sup>1-7</sup> Such studies should be pursued further in search of possible versatile applications. In nature, some phosphoesterases have two metal ions (in most cases, Zn<sup>II</sup> or Mg<sup>II</sup>) at the active sites, and take advantage of their cooperation for the hydrolysis of stable phosphate esters.<sup>8-12</sup> Thus, mimicking the bimetallic synergism should be one of the most promising tactics for the efficient hydrolysis of RNA.

Recently, significant catalytic activities of lanthanide metal ions for RNA hydrolysis were reported.<sup>5,6</sup> Furthermore, their activities were promoted by cooperation with non-lanthanide metal ions.<sup>13,14</sup> The bimetallic cooperation of two lanthanum ions for the hydrolysis of a phosphonate ester was also presented.<sup>15</sup> In these systems, however, at least one of the components must be a lanthanide ion to achieve large catalytic activities. Here we report the first synergetic catalysis by two non-lanthanide metal ions for RNA hydrolysis.† Adenylyl(3'-5')adenosine (ApA) and uridylyl(3'-5')uridine (UpU) are efficiently hydrolysed by the cooperation of Zn<sup>II</sup> ions with Sn<sup>IV</sup>, In<sup>III</sup>, Fe<sup>III</sup> and Al<sup>III</sup> ions, possibly in their hydroxide clusters.

As depicted in Fig. 1(a), ApA is promptly hydrolysed by the combination of ZnCl<sub>2</sub> (10 mmol dm<sup>-3</sup>) and SnCl<sub>4</sub> (10 mmol dm<sup>-3</sup>) at pH 7 (Hepes buffer) and 50 °C. In 3 h, approximately 50% of the ApA is hydrolysed to adenosine (Ado) and its 2'- and 3'-monophosphates (2'A and 3'A). A small amount of 2',3'-cyclic monophosphate of adenosine (A > p) as the hydrolysis intermediate is accumulated. The totally hydrolytic character of the scission is further confirmed by the absence of release of adenine, which should take place if the ribose were cleaved. Quite significantly, both ZnCl<sub>2</sub> and SnCl<sub>4</sub> are virtually inactive when used separately [Fig. 1(b), (c)]. Synergetic catalysis of Zn<sup>II</sup> and Sn<sup>IV</sup> ions, assumed to be the mixed hydroxide cluster, is evident. The pseudo-first order rate constants, *k*, and the magnitude of cooperative acceleration are presented in Table 1.

A continuous variation plot exhibits a steep maximum at the molar fraction 0.5 of Zn<sup>II</sup>. Apparently, the cooperation of the two metal ions occurs in 1:1 molar ratio. UpU is also hydrolysed smoothly by the 1:1 Zn<sup>II</sup>-Sn<sup>IV</sup> combination (the rate constant is 3.0 × 10<sup>-1</sup> h<sup>-1</sup> and the cooperative acceleration is twelve-fold). Direct participation of the nucleic acid bases in the catalysis is unlikely.

Synergetic catalysis is also notable for the Zn<sup>II</sup>-In<sup>III</sup>, Zn<sup>II</sup>-Fe<sup>III</sup> and Zn<sup>II</sup>-Al<sup>III</sup> combinations (Table 1). The Zn<sup>II</sup>-Ni<sup>II</sup>, Zn<sup>II</sup>-Co<sup>II</sup> and Co<sup>II</sup>-Fe<sup>III</sup> combinations exhibit two- to three-fold cooperative acceleration. However, no measurable synergetic effects were observed when Zn<sup>II</sup> was combined with Mg<sup>II</sup>, Ca<sup>II</sup>, Pb<sup>II</sup>, Na<sup>I</sup>, K<sup>I</sup> or Rb<sup>I</sup>. The combinations of Fe<sup>III</sup> with Sn<sup>IV</sup>, Al<sup>III</sup>, Cr<sup>III</sup>, In<sup>III</sup> and Ni<sup>II</sup> (as well as with alkali metal and alkaline earth metal ions) were not cooperative either.

All the above reaction mixtures (in Hepes buffers) involve precipitates, probably of metal hydroxides. However, totally homogeneous solutions of the Zn<sup>II</sup>-Fe<sup>III</sup> combinations can be prepared by use of 100 mmol dm<sup>-3</sup> Tris buffer when [Fe<sup>III</sup>]<sub>0</sub> ≥ [Zn<sup>II</sup>]<sub>0</sub>. In these solutions, the rate constant for the ApA hydrolysis increases almost linearly with [Zn<sup>II</sup>]<sub>0</sub>, when [Fe<sup>III</sup>]<sub>0</sub> is kept constant at 10 mmol dm<sup>-3</sup>. A 1:1 bimetallic cooperation has been shown to occur by the continuous variation method, as

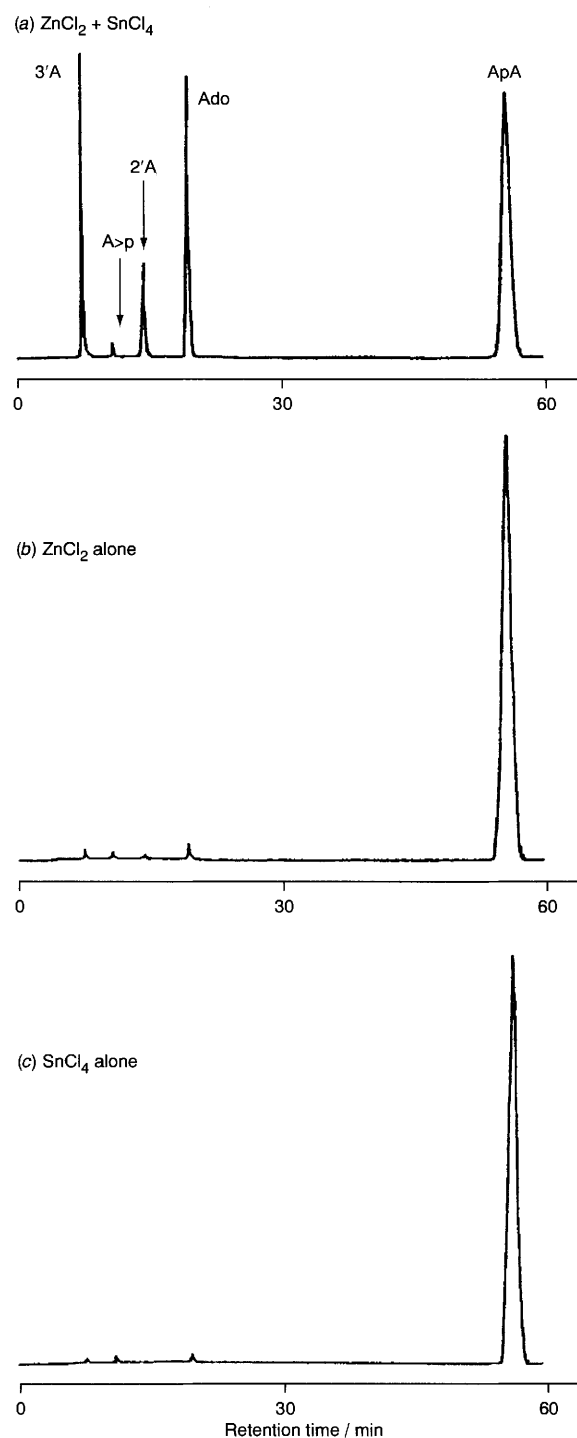


Fig. 1 Reversed-phase HPLC profiles for the hydrolysis of ApA at pH 7 (50 mmol dm<sup>-3</sup> Hepes buffer) and 50 °C for 3 h: (a) ZnCl<sub>2</sub> + SnCl<sub>4</sub>; (b) ZnCl<sub>2</sub> alone; and (c) SnCl<sub>4</sub> alone. The concentration of each of the metal chlorides is 10 mmol dm<sup>-3</sup>.

is the case for the  $Zn^{II}$ - $Sn^{IV}$  systems in Hepes buffers. In addition, the absorbance of  $Fe^{III}$  in the 420–600 nm region gradually decreases with increasing concentration of  $Zn^{II}$ . Formation of a mixed hydroxide cluster from these two metal ions is strongly indicated.<sup>13</sup> Consistently, homogeneous solutions are obtained for the  $Zn^{II}$ - $Al^{III}$  system (even in pH 7 Hepes buffer), although  $Zn^{II}$  rapidly precipitates as polymeric aggregates of the metal hydroxide in the absence of  $Al^{III}$ .

A dideoxynucleotide thymidylyl(3'-5')thymidine was not hydrolysed at reasonable rates at pH 7.5 and 70 °C by any of the metal combinations. Thus, the present RNA hydrolysis proceeds *via* the intramolecular attack by the 2'-hydroxy residue of the ribose towards the phosphorus atom. In one of the most plausible mechanisms, the hydroxide ion bound to  $Zn^{II}$  in the mixed hydroxide cluster promotes the intramolecular attack as a general base catalyst. The reaction is further assisted by the acid catalysis of the water, bound to the  $Fe^{III}$  in the mixed cluster.<sup>‡</sup> The assignment of the two types of catalyses to the metal ions is based on the smaller  $pK_a$  of the  $Fe^{III}$ -bound water than that of the  $Zn^{II}$ -bound water (2.5 vs. 8.2).<sup>16</sup> Similarly, the cooperation in the  $Zn^{II}$ - $Sn^{IV}$  combination can involve both general base catalysis by the  $Zn^{II}$ -bound hydroxide and general acid catalysis by the  $Sn^{IV}$ -bound water ( $pK_a$  -0.6;<sup>16</sup> see Fig. 2). Furthermore, the positive charges of the metal ions electrostatically stabilize the negatively charged transition states for the hydrolysis. The proposed mechanism is consistent with that for the hydrolysis of bis(4-nitrophenyl)phosphate by the  $La^{III}$ - $Fe^{III}$  combination,<sup>13</sup> except for the role of the metal-bound

hydroxide. In the latter catalysis, the  $La^{III}$ -bound hydroxide directly attacks the phosphorus atom as a nucleophile, rather than functioning as a general base catalyst.

The mechanism is supported by the following results. (a) An  $Sn^{IV}$  ion, which is the most eminent for the synergism with  $Zn^{II}$  in Table 1, has the most acidic water of coordination. The  $pK_a$  values of the water molecules on  $In^{III}$ ,  $Fe^{III}$  and  $Al^{III}$  are 2.1–4.4, 2.5–3.1 and 4.3–5.0, respectively.<sup>16</sup> (b) The  $Fe^{III}$ - $Sn^{IV}$  combination shows no synergetic catalysis, in spite of the remarkable catalysis by the  $Zn^{II}$ - $Sn^{IV}$  system. The  $Fe^{III}$ -bound hydroxide is much poorer as a general base catalyst than the  $Zn^{II}$ -bound one, as estimated from the corresponding  $pK_a$  values of the coordination water.

In conclusion, RNA hydrolysis has been achieved for the first time by bimetallic combinations composed of only non-lanthanide metal ions. Detailed study on the mechanism of the cooperation is currently in progress in our laboratory.

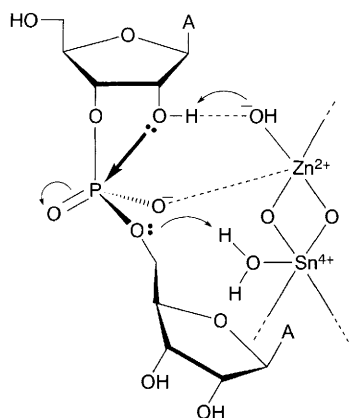
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**Table 1** Cooperation of  $Zn^{II}$  (10 mmol dm<sup>-3</sup>) with  $Sn^{IV}$ ,  $In^{III}$ ,  $Fe^{III}$  and  $Al^{III}$  (10 mmol dm<sup>-3</sup>) for the hydrolysis of ApA at pH 7 and 50 °C<sup>a</sup>

Catalyst	First-order rate constant, $k/10^{-2} h^{-1}$	Magnitude of cooperative acceleration <sup>b</sup>
$Zn^{II} + Sn^{IV}$	21	26
$Zn^{II}$	0.6	
$Sn^{IV}$	0.2	
$Zn^{II} + In^{III}$	14	4.0
$In^{III}$	2.9	
$Zn^{II} + Fe^{III}$	31	3.2
$Fe^{III}$	9.1	
$Zn^{II} + Al^{III}$	4.6	2.6
$Al^{III}$	1.2	

<sup>a</sup> The corresponding metal chlorides were added to 50 mmol dm<sup>-3</sup> Hepes buffers. All the mixtures contained precipitates, probably of the metal hydroxides (see text for detail). <sup>b</sup> Ratio of the rate constant for the catalysis by the combination of metal ions to the sum of the values for each of the metal ions.



**Fig. 2** Proposed mechanism for the hydrolysis of ApA by the  $Zn^{II}$ - $Sn^{IV}$  combination

## Footnotes

<sup>†</sup> Hydrolyses of phosphate esters (other than RNA) by binuclear complexes have been reported.<sup>17</sup> However, it is not clear whether the two metal ions therein in fact cooperate for the hydrolyses or not. Second-order dependence of the hydrolysis rate of 2-hydroxypropyl-*p*-nitrophenylphosphate on a  $Cu^{II}$  complex has also been reported.<sup>18</sup>

<sup>‡</sup> The coordinated water molecules bound to  $Fe^{III}$  can effectively function as acid catalysts during rapid proton-exchange processes, as discussed in ref. 13.

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