Hetero-Dinuclear Metal Complexes for RNA Hydrolysis

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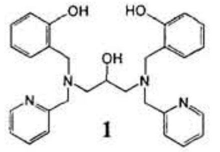
In the presence of a ligand having two metal-binding sites, Fe(III)/Zn(II) and Fe(III)/Cd(II) combinations hydrolyze adenylyl(3'-5')adenosine. The notable activities are ascribed to the synergetic cooperation between the two kinds of metal ions in the hetero-dinuclear complexes.

Design of active catalysts for RNA hydrolysis has been the subject of growing interest.[1] Remarkable catalysis by lanthanide ions and their complexes has been already documented.[2] However, RNA scission by the metal ions, which widely spread in cells (e.g., Zn(II), Fe(II or III), Mg(II), and Ca(II)), has been scarce. These catalysts, if available, should be highly promising for versatile applications.

Recent X-ray crystallography showed that the enzymes for the hydrolysis of phosphoesters often possess two (or more) metal ions at the active sites.[3] Although each of the metal ions is rather poor in the catalytic activity, the cooperation of them gives rise to outstanding activities. The findings strongly indicated that novel catalysts should be obtained by using bimetallic synergism. Consistently, dinuclear Zn(II) complexes for RNA hydrolysis were reported.[4] Other homo-dinuclear complexes (involving two metal ions of the same kind) hydrolyzed phosphoesters.[5] However, little has been known on hetero-dinuclear metal complexes which employ the cooperation of two different metal ions.[6] In the present communication, we report hetero-dinuclear Fe(III)/Zn(II) and Fe(III)/Cd(II) complexes which hydrolyze RNA under mild conditions. Notable synergetic effects are evidenced.



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Dinucleating ligand **1** possessing two phenol residues and two pyridine residues was synthesized, as described by Krebs *et al.*[7] As shown by these authors, this ligand forms a homo-dinuclear Fe(III) complex when $[\mathrm{Fe(III)}]_0/[\mathbf{1}]_0 = 2$. The hydrolysis of adenylyl(3'-5')adenosine (ApA) by all the reaction systems proceeded homogeneously, and was analyzed by reversed-phase HPLC.[8] The metal salts were used as chlorides except for $\mathrm{Zn(NO_3)_2}$. The pH change during the reactions was less than 0.1 unit.

At pH 5.6 and 50 °C, 1:1:1 mixture of Fe(III), Zn(II), and **1** efficiently hydrolyzes ApA (Run 4 in Table 1). The products are adenosine and its 2'- and 3'-monophosphates (2',3'-cyclic monophosphate of adenosine as the intermediate is rapidly hydrolyzed and not much accumulated). In contrast, either 2:1 Fe(III)/**1** mixture or 2:1 Zn(II)/**1** is only poorly active (Runs 2 and 3). Equimolar mixture of Fe(III) and **1** (as well as 1:1 Zn(II)/**1** mixture) is also poor in the activity (Run 1). Bimetallic synergism between Fe(III) and Zn(II) in the present catalyst is conclusive.[9] Quite importantly, 2:1:1 mixture of Fe(III), Zn(II), and **1** is much less active than 1:1:1 Fe(III)/Zn(II)/**1** mixture (compare Run 5 with Run 4). The possibility that free Zn(II) ion cooperates with the 2:1 Fe(III)/**1** complex is ruled out. When another molar Fe(III) ion is added to the 1:1:1 mixture, it occupies the coordination site of **1** for the Zn(II) ion and diminishes the Fe(III)/Zn(II) bimetallic cooperation. When Cd(II) was used in place of Zn(II), a similar synergism with Fe(III)/**1** combination was observed. The rate constant with the 1:1:1 Fe(III)/Cd(II)/**1** mixture ($[\text{each component}]_0 = 2.5$ mmol $\mathrm{dm^{-3}}$) is $3.0 \times 10^{-3}\ \mathrm{h^{-1}}$.

Table 1. The pseudo-first-order rate constants for the hydrolysis of ApA by the combinations of Fe(III), Zn(II), and **1** at pH 5.6 and 50 °C[a]

Run	Concentration / mmol $\mathrm{dm^{-3}}$			Rate constant / $10^{-3}\ \mathrm{h^{-1}}$
	Fe(III)	Zn(II)	**1**	
1	2.5	0.0	2.5	0.2
2	5.0	0.0	2.5	0.2
3	0.0	5.0	2.5	0.3
4	2.5	2.5	2.5	4.1
5	5.0	2.5	2.5	0.7

[a] All the mixtures contain 5 vol.% of acetonitrile.

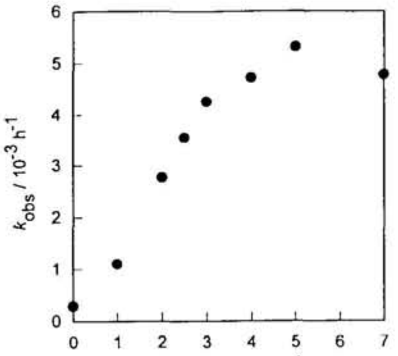

Figure 1. Plot of the pseudo-first-order rate constant for the ApA hydrolysis by Fe(III)/Zn(II)/**1** mixtures as a function of $[\mathrm{Zn(II)}]_0$. Both of $[\mathrm{Fe(III)}]_0$ and $[\mathbf{1}]_0$ are kept constant at 2.5 mmol $\mathrm{dm^{-3}}$ at pH 5.6 and 50 °C.

Figure 1 shows the plot of rate constant for ApA hydrolysis as a function of the feed concentration of Zn(II). Here, $[\mathrm{Fe(III)}]_0$ and $[\mathbf{1}]_0$ are kept constant at 2.5 mmol $\mathrm{dm^{-3}}$. The rate constant significantly increases with increasing $[\mathrm{Zn(II)}]_0$ when $[\mathrm{Zn(II)}]_0$ is rather small, but it shows a gradual saturation. The synergism between Fe(III) and Zn(II) in the presence of **1** has been further substantiated.

As depicted in Figure 2, the pH-rate constant profile for 1:1:1 Fe(III)/Zn(II)/**1** mixture is bell-shaped, showing the maximum around pH 5. The curve satisfactorily fits the mechanism involving both (1) base catalysis by Zn(II)-bound

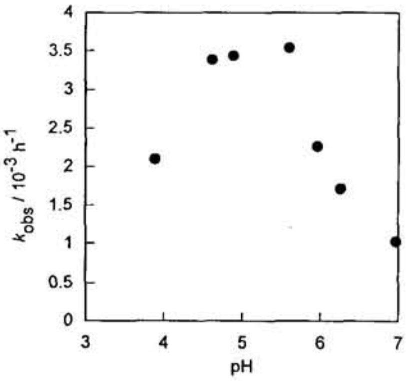

Figure 2. The pH-rate constant profile for the ApA hydrolysis at 50 °C by 1:1:1 mixture of Fe(III), Zn(II), and **1** (the concentration of each is 2.5 mmol dm^{-3}).

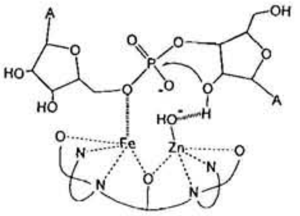

Figure 3. Proposed mechanism for the hydrolysis of ApA by the hetero-dinuclear metal complex.

hydroxide ion and (2) acid catalysis by Fe(III) (or the water bound to it).[10] The former catalysis activates the 2'-OH of the ribose for the nucleophilic attack towards the phosphorus atom, whereas the latter assists the removal of the 5'-OH from the resultant pentacoordinated intermediate (see Figure 3). The acid catalysis can also promote the formation of the intermediate. The possibility that Fe(III) and Zn(II) exchange the roles in the catalysis (base catalysis by the Fe(III)-bound hydroxide ion and acid catalysis by Zn(II)) is unlikely, since then the pH-rate constant profile should take a plateau above the pK_a of the Fe(III)-bound water.

In conclusion, hetero-dinuclear complexes of **1** (a ligand having two metal binding sites) with Fe(III)/Zn(II) and Fe(III)/Cd(II) hydrolyze RNA by bimetallic synergism. Hetero-dinuclear complexes should open a new way to the design of catalysts for phosphoester hydrolysis.

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7 B. Krebs, K. Schepers, B. Bremer, G. Henkel, E. Althaus, W. Müller-Warmuth, K. Griesar, and W. Haase, *Inorg. Chem.*, **33**, 1907 (1994).

8 The mixture of **1** and $FeCl_3$ in methanol was evaporated, and the resultant Fe(III) complex was dissolved in Hepes buffers (50 mmol dm^{-3}). When **1** and the metal salt were directly added to the buffer solutions, it took one day (or even more) to complete the complex formation at room temperature. The hydrolysis of ApA (the initial concentration: 0.05 mmol dm^{-3}) was initiated by the addition of a stock solution of the Fe(III) complex and/or stock solutions of other metal ions to the reaction mixture.

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10 The pK_a values of the Fe(III)-bound and the Zn(II)-bound water are around 3 and 9, respectively (J. Burgess, in "Metal Ions in Solution," Horwood, Chichester (1978)).