Preparation and Study of Dinuclear Zinc(II) Complex for the Efficient Hydrolysis of the Phosphodiester Linkage in a Diribonucleotide

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A dinuclear zinc(II) complex with *N,N,N′,N′*-tetrakis[(2-pyridyl)methyl]-2-hydroxy-1,3-diaminopropane efficiently hydrolyses ApA [adenylyl(3′-5′)adenosine] at pH 7 and 50 °C; the complex can thus be regarded as a good artificial ribonuclease which effectively mimics enzyme active sites.

Much effort has been focused on developing efficient catalysts for the hydrolysis of nucleic acids.[1–3]

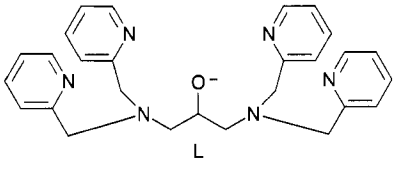
Many enzymes such as alkaline phosphatase, phospholipase C and P1 nuclease contain two or more Zn^{II} ions in the active site, which are believed to play important roles in the hydrolysis of the phosphomonoester or phosphodiester bonds.[4] It has also been suggested that two Mg^{II} ions participate cooperatively in RNA scission by hammerhead ribozymes.[4d,5] These observations indicate that suitable design of a bimetal complex system will provide an efficient catalyst for the hydrolysis of nucleic acids.[6–10]

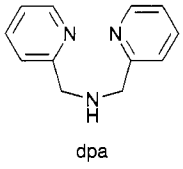
Here we report that a dinuclear zinc(II) complex with *N,N,N′,N′*-tetrakis[(2-pyridyl)methyl]-2-hydroxy-1,3-diaminopropane, L,[11],† hydrolyses a diribonucleotide efficiently under mild conditions. The ligand L contains two bis[(2-pyridyl)methyl]amine (dpa) moieties which can bind Zn^{II} with a high stability.[12] The hydrolysis activity of the dinuclear zinc(II) complex is significantly greater than that for the corresponding mononuclear analogue.

Stable complex formation of $ZnCl_2$ with L is clearly evidenced by ^1H NMR spectroscopy {270 MHz, D_2O, pD 7.0, room temp., L = 5 mmol dm^{-3}, [$ZnCl_2$] = 0–12.5 mmol dm^{-3}}. Depending on the [$ZnCl_2$]/[L] ratio (r), two independent species (**1** and **2**) are formed.‡ When $r < 1$, signals of a new species **1** are observed in addition to signals due to free L. At $r = 1$, the signals due to free L completely disappear and only those of **1** are detected. On further addition of $ZnCl_2$, signals due to another species **2** appear and at ratios $r > 2$, **2** is the sole complexed species. Apparently, **2** is a 2 : 1 complex of Zn^{II} and L, and **1** is a 1 : 1 complex.

Hydrolysis of a ribonucleotide dimer, adenylyl(3′-5′)adenosine (ApA), by the $ZnCl_2$–L complex system was conducted at 50 °C, pH 7.0 {Hepes buffer = 50 mmol dm^{-3}, $[ApA]_0$ = 0.1 mmol dm^{-3}, [$ZnCl_2$] = 5 mmol dm^3, [L] = 0–10 mmol dm^{-3}} and was followed by reversed phase ODS HPLC. The products are adenosine, its 2′- and 3′-phosphates, and the 2′,3′-cyclic phosphate, as usually observed for metal-assisted hydrolysis of ApA.[2a] The reactions satisfactorily showed pseudo-first-order kinetics.

Results are shown in Fig. 1. The rate of hydrolysis largely depends on the [$ZnCl_2$] : [L] ratio r. In the absence of L, a precipitate of $Zn(OH)_2$ is formed, and hydrolysis of ApA is essentially negligible. In contrast, solutions containing L are completely homogeneous. The hydrolysis rate increases substantially and monotonically with increasing concentration of L (at $r \geq 2$), the maximum rate being observed at $r = 2$. Significantly, further increase of [L] results in a dramatic suppression of the hydrolysis. Since hydrolysis is virtually negligible when $r \leq 1$ ([L] $\geq$ 5 mmol dm^{-3}) it is apparent that the dinuclear complex **2** is the active species, while the mononuclear complex **1** and the free ligand L are inactive.





Therefore, hydrolysis of ApA is substantially accelerated by the cooperation of two Zn^{II} ions in the dinuclear complex.§ Consistently, the $ZnCl_2$-tpa complex {tpa = tris[(2-pyridyl)-methyl]amine}, the mononuclear analogue of $ZnCl_2$–L, is inactive for ApA hydrolysis under the same conditions.

The role of metal ions in hydrolysis should be (*i*) to promote the deprotonation of 2′-OH, (*ii*) to stabilize the five-coordinate phosphorus intermediate, and (*iii*) to facilitate the removal of the 5′-O^- from the intermediate. Binding of two metal ions to ApA should promote these processes efficiently. It may be also possible that some of these steps are catalysed by water molecules or hydroxide ions bound to Zn^{II}. A proposed mechanism is shown in Fig. 2. Two Mg^{II} ions play a similar role in the RNA scission by hammerhead ribozymes.[5]

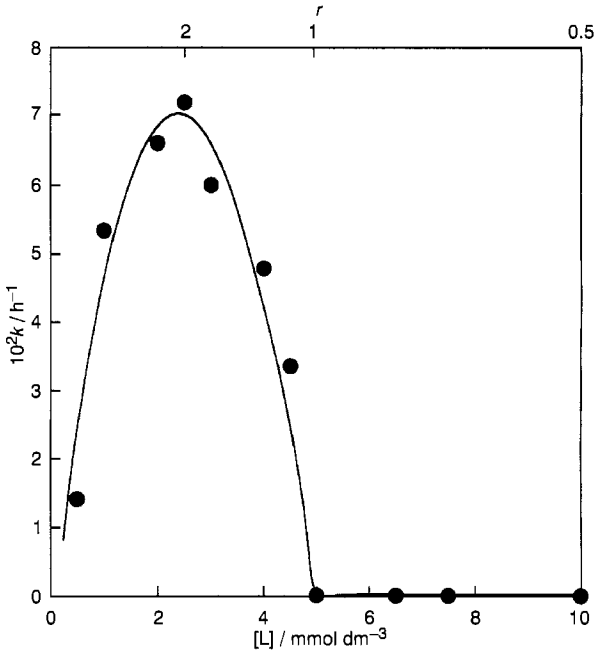


Fig. 1 Dependence of the rate of ApA hydrolysis on the concentration of L [$ApA]_0$ = 0.1 mmol dm^{-3}, [$ZnCl_2$] = 5 mmol dm^{-3}, 50 °C, pH 7.0 (Hepes buffer, 50 mM)

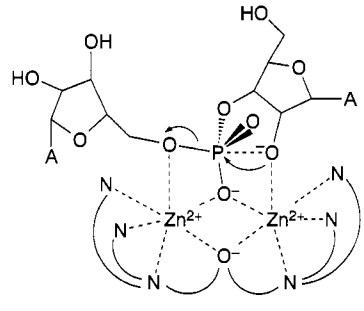


Fig. 2 Proposed mechanism for the hydrolysis of ApA by **2**. For simplification, only donor atoms of L are shown.

Participation of two metal ions in RNA hydrolysis has been suggested by the hydrolysis of model compounds of RNA by copper(II) complexes,[7] or by the hydrolysis of a specially designed substrate which can bind two La^{III} ions.[8] X-Ray crystallographic studies have shown that two Zn^{II} ions bind to phosphoesters.[10a,b] The present results demonstrate direct evidence for the effective cooperation of two Zn^{II} ions in the hydrolysis process.

In conclusion, the dinuclear zinc(II) complex is very effective for the hydrolysis of a diribonucleotide. Complex systems of this type are important not only for mimicking the active centres of enzymes, but also for the design of active sites of artificial ribonucleases.

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Footnotes

† Ligand L is known to form dinuclear complexes with $Mn^{II}Mn^{III}$,[11a] Co^{III}[11b] and Fe^{II}.[11c]

‡ ^{1}H NMR spectral data (270 MHz, D_2O, pD 7, room temp.) L: δ 2.46 (2 H, dd, *J* 14, 8 Hz), 2.60 (2 H, dd, 14, 5 Hz), 3.58–3.68 (1 H, m), 3.79 (8 H, s), 7.30 (4 H, t, 6 Hz), 7.38 (4 H, d, 8 Hz), 7.76 (4 H, t, 8 Hz), 8.37 (4 H, d, 5 Hz). **1**: 3.07 (2 H, br), 3.27 (2 H, br), 4.19–4.48 (9 H, m), 7.43 (4 H, br), 7.50 (4 H, br), 7.96 (4 H, br), 8.41 (4 H, br). The broad signals of **1** can be ascribed to relatively slow exchange of donor atoms of L and the linewidths are temperature dependant. **2**: 2.09 (2 H, dd, 13, 11 Hz), 2.91 (2 H, dd, 13, 2 Hz), 3.40 (1 H, br), 4.05 (4 H, AB pattern, J_{AB} 17 Hz, δν 58 Hz), 4.14 (4 H, s), 7.32 (2 H, d, 8 Hz), 7.37 (2 H, d, 8 Hz), 7.39 (2 H, t, 6 Hz), 7.46 (2 H, t, 6 Hz), 7.88 (4 H, t, 8 Hz), 8.54 (2 H, d, 5 Hz), 8.63 (2 H, d, 5 Hz).

§ The rate of hydrolysis (log *k*) by **2** shows linear dependence with pH below 7.5, and reaches a plateau in the higher pH region 8–9.

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