

Efficient and unique cooperation of three zinc(ii) ions in the hydrolysis of diribonucleotides by a trinuclear zinc(ii) complex

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A trinuclear Zn²⁺ complex is prepared using a ligand having six pyridine moieties, *N,N,N',N',N'',N''*-hexakis(2-pyridylmethyl)tris(2-aminoethyl)amine (L³), and efficiently hydrolyses diribonucleotides at pH 7 and 50 °C, showing much greater activity than a dinuclear Zn₂L² complex.

A number of enzymes for phosphoester hydrolysis have two or three Zn²⁺ ions in the catalytic centre.¹ Therefore, polynuclear metal complexes are expected to be useful as catalysts for the hydrolysis of nucleic acids. We have recently reported that a dinuclear zinc(ii) complex with *N,N,N',N'*-tetrakis(2-pyridylmethyl)-2-hydroxy-1,3-diaminopropane (L², Fig. 1) efficiently hydrolyses a diribonucleotide, the high activity arising from the cooperation of two Zn²⁺ ions.²

It is important to elucidate the cooperation effect of three zinc(ii) ions in phosphoester hydrolysis, since three Zn²⁺ ions have been suggested to participate in enzymatic reactions.^{1b} Thus, a new ligand L³ was designed in this study.[†] L³ is expected to bind three Zn²⁺ ions, since each of the three dpa (L¹) units in L³ should bind to Zn²⁺ [dpa = bis(2-pyridylmethyl)amine].³ Here, we report that the trinuclear complex Zn₃L³ hydrolyses diribonucleotides much more efficiently than the dinuclear Zn₂L² complex.

The trinuclear zinc(ii) complex was prepared in an aqueous solution by mixing Zn(NO₃)₂ and L³ in a molar ratio of 3 : 1, and was characterized by ¹H NMR spectroscopy. A mixture of Zn(NO₃)₂ (15 mM) and L³ (5 mM) in D₂O (pD 7) gave a very

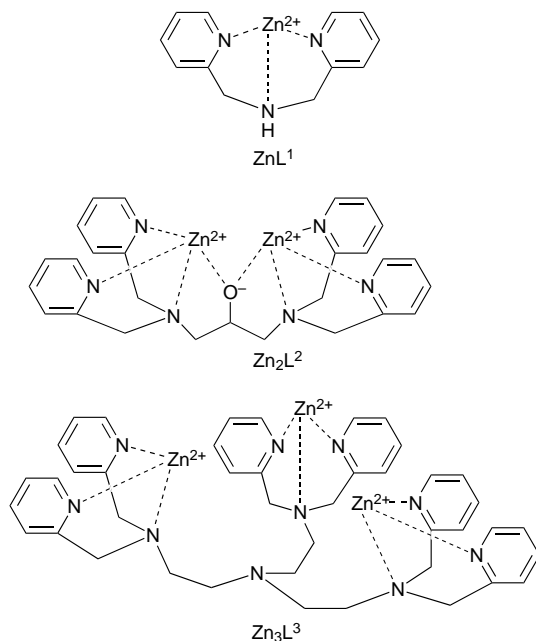


Fig. 1 Structures of ligands. The proposed coordination modes of Zn²⁺ are also shown. The drawn structures of the complexes are only partial, and additional molecules coordinating at the remaining sites of Zn²⁺ are not specified.

simple ¹H NMR spectrum;‡ the six 2-pyridylmethyl moieties in L³ were observed as one set of sharp signals, indicating the formation of a complex in which all the 2-pyridylmethyl moieties are equivalent. In contrast, when the concentration of Zn(NO₃)₂ was < 15 mM, the ¹H NMR spectrum gave complicated signals, corresponding to the formation of several complex species having lower symmetries. These observations indicate the formation of a trinuclear complex having three-fold symmetry in a 3 : 1 Zn(NO₃)₂-L³ mixture (Fig. 1).

Hydrolysis of ribonucleotide dimers, NpN', by Zn₃L³ was conducted at 50 °C and pH 6.9 (50 mM Hepes buffer).§ The reactions were followed by reversed-phase ODS HPLC. The products were nucleoside (N'), 3'- and 2'-monophosphates (3'-, 2'-NMP), and the 2',3'-cyclic monophosphate (2',3'-cNMP).¶ The reactions showed satisfactory pseudo-first-order kinetics.

The rate of NpN' hydrolysis by the Zn(NO₃)₂-L³ complex system is largely dependent on the metal ion-to-ligand ratio. Fig. 2 shows the rate constants for ApA hydrolysis at various concentrations of L³ and a constant Zn²⁺ concentration {[Zn(NO₃)₂] = 15 mM}. The maximal rate was observed at a ratio [Zn(NO₃)₂] : [L³] = 3 : 1 indicating that the phosphoester hydrolysis is primarily promoted by the trinuclear Zn₃L³ complex.

The results of hydrolyses of various diribonucleotides by the trinuclear complex are listed in Table 1 and for comparison, equivalent data for the dinuclear Zn₂L² complex are also listed. The trinuclear complex hydrolyses diribonucleotides efficiently, showing a considerable dependence on the structure of the substrate. For all the substrates examined, the activity of the trinuclear complex is much greater than that of the dinuclear complex. The half-life of CpA hydrolysis by the trinuclear Zn₃L³ complex is 16 min (*cf.* 3 h for the dinuclear Zn₂L² complex).

Further, the trinuclear Zn₃L³ complex-catalysed hydrolysis is unique in product formation with 3'-NMP preferentially formed

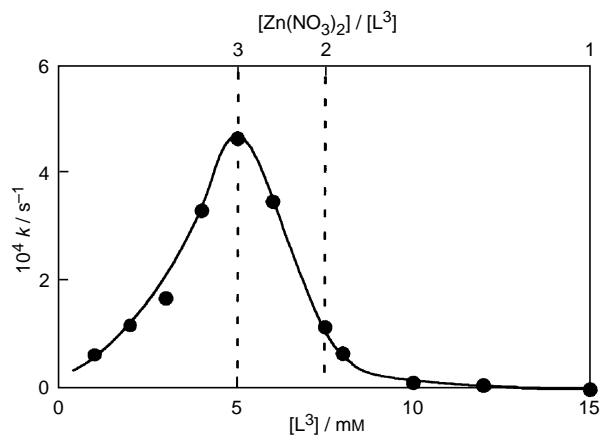


Fig. 2 Rate constants for ApA hydrolysis with various concentrations of L³ at a constant Zn²⁺ concentration {[Zn(NO₃)₂] = 15 mM} at pH 6.9 and 50 °C

over 2'-NMP. In the hydrolysis of CpA, ApA, GpA and GpC, the formation ratio of 3'-NMP to 2'-NMP is > 90%. These results are significant, since 3'-NMP/2'-NMP selectivities are low in hydrolysis by Zn₂L².

Trinuclear metal centres have been found in crystals of P1 nuclease,^{1a} phospholipase C,^{1b} and alkaline phosphatase.^{1c} On the basis of their crystal structures, participation of three Zn²⁺ ions in the catalytic process has been proposed.^{1b} Although cooperative effects of two metal ions in phosphoester hydrolyses have been directly observed by using dinuclear complexes,^{2,4} the cooperation of three metal ions in the catalytic process has not yet been clarified. The present results clearly show the efficiency of the trinuclear zinc(ii) cluster in phosphoester hydrolysis.

In conclusion, a novel trinuclear zinc(ii) complex is prepared using L³. Because of the high efficiency and the selective formation of the 3'-monophosphate, the trinuclear zinc(ii) complex is a unique catalyst for the hydrolysis of RNA.

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Table 1 Hydrolysis of diribonucleotides (NpN') by polynuclear zinc(ii) complexes at pH 6.9 and 50 °C^a

Substrate	10 ⁵ k/s ⁻¹	t _{1/2} /s	3'-NMP:2'-NMP
Zn₃L³			
CpA	72	960	> 9:1
ApA	46	1400	9:1
GpA	26	2700	9:1
UpA	16	4500	7:3
ApG	17	4100	8:2
GpC	8	8500	9:1
Zn₂L²			
CpA	6	11 000	4:6
ApA	5	15 000	6:4
GpA	5	15 000	6:4
UpA	6	12 000	6:4
ZnL¹			
ApA	— ^b	— ^b	

^a [Zn(NO₃)₂] = 15 mM, [L³] = 5 mM or [L²] = 7.5 mM, [NpN'] 0 = 0.1 mM. ^b No reaction after 24 h.

Footnotes

† L³ was prepared from tris(2-aminoethyl)amine and 2-picolylaldehyde in methanol, with subsequent reduction by NaBH₃CN at room temp. and was isolated as a perchlorate salt.

CAUTION: although the perchlorate salt is moderately stable, it is a potential hazard and should therefore be handled with care and in small quantities. Anal. Calc. for C₄₂H₅₄Cl₆N₁₀O₂₄: C, 38.93; H, 4.20; N, 10.81. Found: C, 38.60; H, 4.37; N, 10.53%. ¹H NMR (270 MHz, D₂O) δ 2.93 (6 H, t, J 6.5 Hz), 3.20 (6 H, t, 6.5), 3.79 (12 H, s), 7.30 (6 H, d, 7.8), 7.32 (6 H, t, 7.8), 7.77 (6 H, t, 7.8), 8.34 (6 H, d, 7.8).

‡ ¹H NMR (270 MHz, D₂O, 50 °C) δ 2.91 (6 H, br), 2.94 (6 H, br), 4.17 (12 H, s), 7.57 (6 H, d, 7.8), 7.64 (6 H, t, 7.8), 8.15 (6 H, t, 7.8), 8.53 (6 H, d, 7.8).

§ Diribonucleotides, CpA, ApA, GpA, UpA, ApG and GpC, were purchased from Sigma.

¶ 2',3'-cNMP is readily hydrolysed to 3'- and 2'-NMP, and is observed as an intermediate.

|| Participation of three Zn²⁺ in the phosphoester hydrolysis has been also suggested on the basis of the structure of a complex consisting of an inorganic phosphate and three mononuclear zinc(ii) complexes (T. Koike, S. Kajitani, I. Nakamura, E. Kimura and M. Shiro, *J. Am. Chem. Soc.*, 1995, **117**, 1210).

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