Dinuclear Copper(II) Complex That Hydrolyzes RNA

Mary Jane Young and Jik Chin*

Department of Chemistry, McGill University
Montreal, Canada H3A 2K6

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In nature there are many phosphoesterases that are activated by two or more metal ions. They include the 3′,5′-exonuclease[1] from the Klenow fragment of DNA polymerase I, RNase H[2] from HIV reverse transcriptase, P1 nuclease,[3] alkaline phosphatase,[4] and phospholipase C.[5] *Tetrahymena* ribozyme[6] is also thought to be activated by at least two metal ions. Over the years interesting dinuclear Co(III),[7−9] Cu(II),[10,11] and La(III)[12] complexes that hydrolyze activated phosphate esters have been reported. An unactivated phosphonate ester that can coordinate two La(III) ions has also been shown to hydrolyze rapidly.[13] Here we compare the reactivity of a mononuclear (**1**) and a dinuclear (**2**) Cu(II) complex for hydrolyzing RNA (Scheme 1).

A novel organic ligand (**L**) that can bind two metal ions was prepared by reacting 2 equiv of the 1,4,7-triazacyclononane orthoamide[14] with 1,8-bis(bromomethyl)naphthalene in DMSO followed by base workup (Scheme 1).[15] The $CuCl_2$ complexes of **L** and 1,4,7-triazacyclononane were prepared by mixing ethanolic solutions of the organic ligand and $CuCl_2$.[15] The metal-bound chlorides in **1** and **2** are expected to be displaced by water molecules in aqueous solvents. Potentiometric titration of **1** gave 1 equiv of titratable proton around pH 7 while **2** gave 2 equiv of titratable protons around pH 6. It is well-known that hexaamine ligands with flexible backbones can effectively encapsulate a single metal ion rather than coordinate two metal ions.[16] Potentiometric titrations of mixed solutions (supporting information) of $CuCl_2$ and the free ligand (**L**) in ratios of 0:1, 1:0, 1:1, and 2:1 demonstrate that **L** binds both copper ions in water when 2 equiv of $CuCl_2$ is added.

Cleavage of ApA to adenosine, 2′-AMP, and 3′-AMP as well as cleavage of 2′,3′-cAMP to 2′-AMP and 3′-AMP was monitored by HPLC.[17] All of the reaction solutions were buffered with HEPES or MES (10 mM). A typical HPLC plot for **2** (2 mM)-promoted cleavage of ApA (0.05 mM) to completion at pH 6.0 and 50 °C is shown in Figure 1. The concentration of 2′,3′-cAMP does not accumulate during the cleavage reaction. It is evident from the HPLC plot that the cleavage reaction occurs hydrolytically, producing adenosine, 3′-AMP, and 2′-AMP. A typical HPLC plot for **2** (2 mM)-promoted cleavage of 2′,3′-cAMP (0.05 mM) to completion at pH 6.0 and 25 °C is shown in Figure 2. The optimal reactivity of **1** for cleaving ApA and 2′,3′-cAMP is reached at about pH 7.3 while that of **2** for cleaving the same substrates is reached

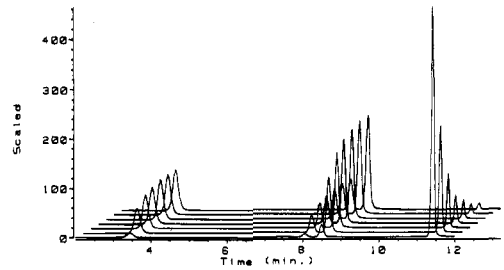


Figure 1. HPLC traces of **2** (2 mM)-promoted cleavage of ApA (0.05 mM) at 50 °C, pH 6.0 (10 mM MES). Retention times are as follows: 3′-AMP = 3.5 min; 2′,3′-cAMP = 7.5 min; 2′-AMP = 8 min; adenosine = 8.5 min; ApA = 11.4 min. Reaction times are from foreground to background, 20, 100, 180, 260, 340, 420, and 500 min.

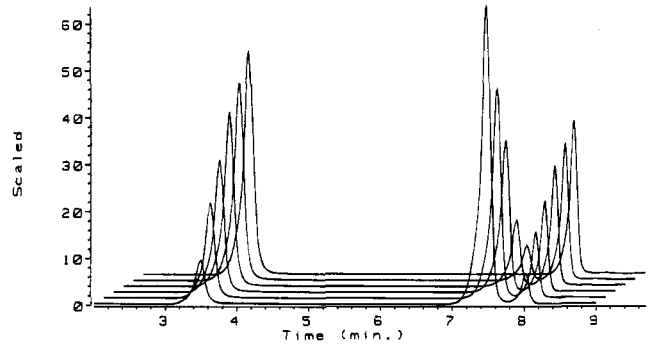
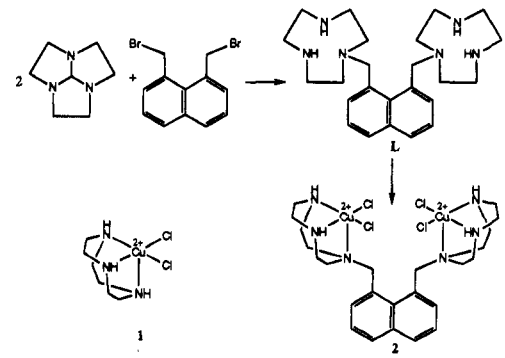


Figure 2. HPLC traces of **2** (2 mM)-promoted cleavage of 2′,3′-cAMP at 25 °C, pH 6.0 (10 mM MES). Retention times are as follows: 3′-AMP = 3.5 min; 2′,3′-cAMP = 7.5 min; 2′-AMP = 8 min. Reaction times are from foreground to background, 1, 3, 5, 10, 15, and 60 min.

Scheme 1



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(15) Characterization of **L**: ^{1}H NMR ($CDCl_3$, TMS) δ 2.27 (8H, t), 2.65 (16H, m), 4.42 (4H, s), 7.35 (2H, t), 7.52 (2H, d), 7.79 (2H, d); ^{13}C NMR ($CDCl_3$, dioxane (66.7 ppm)) δ 46.1, 46.3, 52.7, 63.5, 123.7, 128.6, 129.6; HRMS (EI, 70 eV, direct probe 100 °C) *m/z* ($M^{\bullet+}$) calcd for $C_{24}H_{38}N_6$ 410.3152, found 410.3158. Anal. Calcd for $C_{24}H_{38}N_6Cu_2Cl_4{\cdot}H_2O$ (**2**): C, 41.33; H, 5.78; N, 12.05. Found: C, 41.51; H, 5.77; N, 11.77.
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(17) Aliquots of the reaction mixture for cleavage of ApA and 2′,3′-cAMP were analyzed with a HP1090 HPLC using a 100 × 2.1 mm C-18 column (5 μm Hypersil at 40 °C) running 0.5 mL/min of 0.2 M $NH_4H_2PO_4$ buffer (pH 5.5) for the first 5 min followed by a linear gradient to 50% of a 60/40 methanol/water solution over the next 10 min. Aliquots of the reaction mixture for **2**-promoted cleavage of 2′,3′-cAMP were first quenched with 100 mM EDTA solutions prior to analysis by HPLC. The rate constants for **1**-promoted cleavage of ApA and 2′,3′-cAMP were obtained by the initial rate method while those for **2**-promoted cleavage of the diesters were obtained by fitting the first three half-lives of the reaction according to the first-order kinetics equation.

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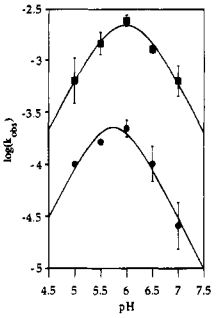


Figure 3. pH−rate profiles for **2** (2 mM)-promoted cleavage of (top) 2′,3′-cAMP at 25 °C and (bottom) ApA (0.05 mM) at 50 °C.

Table 1. Pseudo-First-Order Rate Constants (s^{-1}) for Cleavage of ApA (0.05 mM) and 2′,3′-cAMP (0.05 mM) Promoted by **1** (4 mM) and **2** (2 mM)

	ApA[a]	2′,3′-cAMP[a]
1[b]	4.2×10^{-7}	8.7×10^{-6}
2[b]	2.2×10^{-4}	2.5×10^{-3}

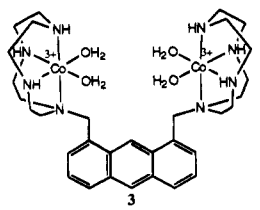
[a] The reactions of ApA and 2′,3′-cAMP were monitored at 50 and 25 °C, respectively. [b] The reaction solutions of **1** and **2** were buffered at pH 7.3 (10 mM HEPES) and pH 6.0 (10 mM MES), respectively.

at about pH 6.0 (Figure 3). The pH−rate profiles (Figure 3) were fitted according to eq 1,[18] where $[\mathbf{2}]_T$ is the total concentration of the dinuclear metal complex, [H] is the proton concentration, K_{a1} and K_{a2} are the first and second acid dissociation constants for **2**, and k is the second-order rate constant for the monohydroxy form of **2**-promoted cleavage of ApA and 2′,3′-cAMP.[19] The dinuclear complex (**2**) is several hundred times more reactive than the mononuclear complex (**1**) per metal center for cleaving ApA or 2′,3′-cAMP (Table 1).

$$k_{obs} = k[\mathbf{2}]_T/(1 + [H]/K_{a1} + K_{a2}/[H]) \quad (1)$$

It has been shown that **1** slowly hydrolyzes an activated phosphate diester (bis(*p*-nitrophenyl) phosphate).[20] The reactivity of **1** is also low for cleaving ApA and 2′,3′-cAMP (Table 1). We reasoned that a dinuclear metal complex that can provide double Lewis acid activation by bridging phosphate diesters should be much more reactive for cleaving RNA than mononuclear metal complexes[21] that provide single Lewis acid activation by coordinating only one of the phosphoryl oxygens. A dinuclear Cu(II) complex that can bridge phosphate diesters has recently been shown to cleave an activated RNA model (2-hydroxypropyl *p*-nitrophenyl phosphate) with unprecedented reactivity.[10] However, the dinuclear Cu(II) complex is not reactive for cleaving RNA itself. It may be that the dinuclear Cu(II) complex is sterically too bulky to bridge the RNA phosphate diester.

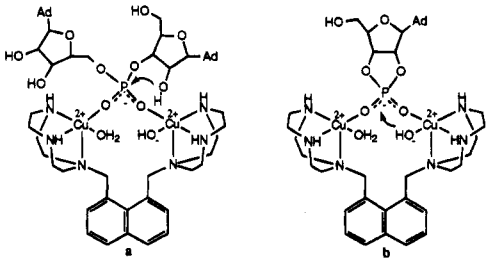
In an interesting study, Sargeson *et al.* showed that there is some cooperativity between two Co(III) centers for hydrolyzing an activated phosphate monoester.[7] In another interesting study, Czarnik and Vance[8] showed that there is cooperativity between the two metal centers in **3** for hydrolyzing a phosphate monoester but not for hydrolyzing a phosphate diester. In the above studies,[7,8] the second metal gave 10−50-fold rate acceleration for hydrolyzing the phosphate monoester.



3

The structure of our dinuclear Cu(II) complex **2** closely parallels that of dinuclear Co(III) complex **3**. Interestingly, **2** is about 300−500 times more reactive than **1** per metal center for cleaving 2′,3′-cAMP and ApA. Although **2** is not the most reactive metal complex reported to date for hydrolyzing RNA,[22] it represents the first example of a simple dinuclear metal complex whose metal centers cooperatively cleave the phosphate diester bonds of RNA and 2′,3′-cAMP. We propose that the cooperativity is due to bridging of the phosphate esters (Scheme 2) as in previous systems.[7,8,10] Formation of both 3′-AMP and 2′-AMP in the cleavage of ApA (Figure 1) indicates that the 2′-hydroxyl group is an intramolecular nucleophilic catalyst (Scheme 2a). The bell-shaped pH−rate profiles (Figure 3) indicate that the monohydroxy form of the dinuclear complex or its kinetic equivalent is the active species for cleaving ApA and hydrolyzing 2′,3′-cAMP. The copper-hydroxide may act as a general base catalyst in both Scheme 2a and 2b or as a nucleophilic catalyst in Scheme 2b.

Scheme 2



The second-order rate constants for hydroxide-catalyzed hydrolysis of ApA at 60 °C and 2′,3′-cAMP at 30 °C are 3.3×10^{-2} M^{-1} s^{-1} and 1.5×10^{-3} M^{-1} s^{-1}, respectively.[23,24] Compared to the background hydroxide rate at pH 6, **2** (2 mM) provides about 5 orders of magnitude rate acceleration for cleaving ApA and 8 orders of magnitude rate acceleration for hydrolyzing 2′,3′-cAMP. The large differential in the rate acceleration for **2**-promoted cleavage of ApA and 2′,3′-cAMP (10^5 vs 10^8) suggests that the metal−hydroxide in Scheme 2b is acting as a nucleophilic catalyst rather than as a general base catalyst.

In conclusion, **2** is a novel dinuclear Cu(II) complex that for the first time shows cooperativity between two metal centers for cleaving RNA. The dinuclear Cu(II) complex **2** is about 500 times more reactive per metal center than the mononuclear Cu(II) complex **1** for cleaving ApA. Furthermore, the dinuclear complex is about 300 times more reactive per metal center than the mononuclear complex for hydrolyzing 2′,3′-cAMP. Interestingly, the simple dinuclear complex provides an enormous rate acceleration for hydrolyzing 2′,3′-cAMP over the background hydroxide rate at pH 6.

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Supporting Information Available: Potentiometric titrations of mixed solutions of $CuCl_2$ and the free ligand (**L**) demonstrating that **L** binds both copper ions in water when 2 equiv of $CuCl_2$ is added (2 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be dowloaded from the Internet, see any current masthead page for ordering information and Internet access instructions.

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(19) Kinetic experiments were carried out at least in triplicate. The error bars represent ±1 standard deviation. For ApA, $K_{a1} = 7.9 \times 10^{-7}$; $K_{a2} = 4.0 \times 10^{-6}$; $k = 5.9 \times 10^{-1}$ M^{-1} s^{-1}. For 2′,3′-cAMP, $K_{a1} = 1.0 \times 10^{-6}$; $K_{a2} = 1.0 \times 10^{-6}$; $k = 3.4$ M^{-1} s^{-1}.
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