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Trinuclear Copper(II) Complex Showing High Selectivity for the Hydrolysis of 2′−5′ over 3′−5′ for UpU and 3′−5′ over 2′−5′ for ApA Ribonucleotides

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Abstract: The cooperative action of multiple Cu(II) nuclear centers is shown to be effective and selective in the hydrolysis of 2′−5′ and 3′−5′ ribonucleotides. Reported herein is the specific catalysis by two trinuclear Cu(II) complexes of **L3A** and **L3B**. Pseudo first-order kinetic studies reveal that the **L3A** trinuclear Cu(II) complex effects hydrolysis of Up(2′−5′)U with a rate constant of 28×10^{-4} min^{-1} and Up(3′−5′)U with a rate constant of 0.5×10^{-4} min^{-1}. The hydrolyses of Ap(3′−5′)A and Ap(2′−5′)A proceed with rate constants of 24×10^{-4} min^{-1} and 0.5×10^{-4} min^{-1} respectively. The **L3A** trinuclear Cu(II) complex demonstrates high specificity for Up(2′−5′)U and Ap(3′−5′)A. Similar studies with the more rigid **L3B** trinuclear Cu(II) complex shows no selectivity and yields lower rate constants for hydrolysis. The selectivity observed with the **L3A** ligand is attributed to the geometry of the ligand-bound diribonucleotide which ultimately dictates the proximity of the attacking hydroxyl and the phosphoester to a Cu(II) center for activation and subsequent hydrolysis.

Introduction

Interest in the hydrolysis of RNA has been rapidly increasing,[1] and notable catalysis using lanthanide ions and their complexes has been documented.[2] Furthermore, a number of dinuclear[3,4] and trinuclear [5] metal complexes have been reported, thereby demonstrating that the hydrolysis of RNA can be promoted by cooperation between multiple metallic centers.[6] However, metal complexes showing sufficiently high substrate-specificity in RNA hydrolysis have been scarce, with only one reported base-selective cleavage of 3′−5′ diribonucleotides by Hamilton.[7] Such complexes likely require many sites for substrate recognition.

Herein, we report that the trinuclear Cu(II) complex of *N*,*N*,*N*′,*N*′,*N*″,*N*″-hexa[(2-pyridyl)methyl]-1,3,5-tris(aminomethyl)benzene (**L3A**) shows remarkable substrate-specificity in the hydrolysis of Up(2′−5′)U compared to Up(3′−5′)U and in the hydrolysis of Ap(3′−5′)A compared to Ap(2′−5′)A.[8] The catalytic activity is not only strongly dependent on the kind of phosphodiester linkage, but also on the sequence. The catalysis by trinuclear Cu(II) complex of *N*,*N*,*N*′,*N*′,*N*″,*N*″-hexa[(2-pyridyl)methyl]-1,3,5-tris(aminomethyl)-2,4,6-triethylbenzene (**L3B**), which has restricted molecular flexibility compared to that of the **L3A** complex, is also reported. The origin of substrate-specificity is discussed in terms of kinetic and spectroscopic results.

Results and Discussion

Synthesis of the Ligands L3A and L3B. The structures of the ligands used in this study are presented in Scheme 1.

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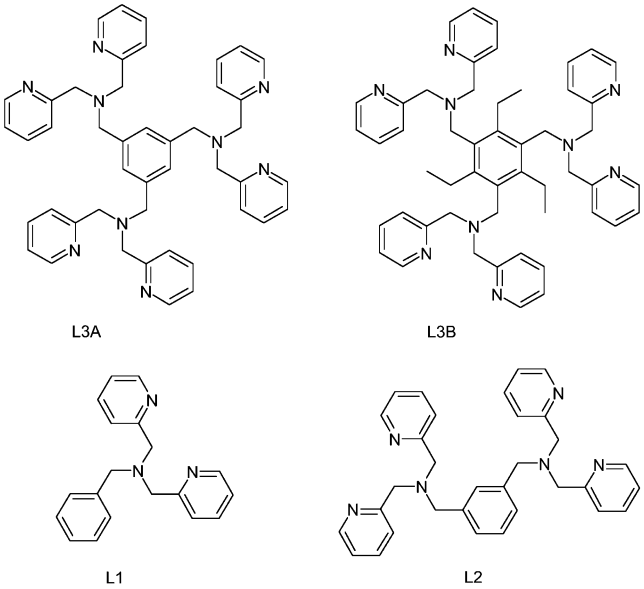
Scheme 1. Ligands Used in the Present Study



Trinuclear ligand **L3A** was prepared from 1,3,5-tris(bromomethyl)benzene and 2,2′-dipicolylamine. Tris(bromomethyl)benzene was prepared by irradiating UV light on a mixture of 1,3,5-trimethylbenzene, N-bromosuccinimide, and benzoyl peroxide in carbon tetrachloride.[9] Subsequently, tris(bromomethyl)benzene was treated with 2,2′-dipicolylamine, and *N*,*N*-diisopropylamine in acetonitrile. This yielded the desired **L3A** ligand. Dinuclear ligand **L2** was synthesized in a fashion similar to **L3A**, using 1,3-di(bromomethyl)benzene and 2,2′-dipicolylamine. The **L3B** was prepared by combining 1,3,5-tris(chloromethyl)-2,4,6-triethylbenzene,[10] 2,2′-dipicolylamine and potassium carbonate in dry acetonitrile.

Formation of Trinuclear Cu(II) Complexes of L3A and L3B. When **L3A** was added to an aqueous solution of Cu(II) at pH 7.0, a new absorption band appeared between 550 and 800 nm. The absorption maximum λ_{max} was 662 nm. As the concentration of **L3A** was increased, ($[Cu(II)]_0$ was kept constant at 1.2 mM), the absorbance for this band linearly increased until $[Cu(II)]_0/[\mathbf{L3A}]_0 = 3$, at which point a plateau was attained. A clear break was observed at the mole ratio 3. Virtually the same results were obtained for the Cu(II)/**L3B** system ($\lambda_{max} = 674$ nm). The trinuclear Cu(II) complexes, of both the **L3A** and the **L3B** ligands, were formed almost quantitatively in aqueous solutions. According to a potentiometric titration, the pK_a values of the metal-bound water ligands in the Cu(II)/**L3A** complex are 6.9, 7.4, and 7.8, respectively. These values are close to each other, and comparable with the corresponding values for analogous Cu(II) complexes.[11] Apparently all of the three Cu(II) ions in this complex are in similar environments, and the metals act independently in the pH titrations.

The X-ray structure of a trinuclear Cu(I) complex of **L3B** has previously been reported.[12] The result of the X-ray crystallography on the trinuclear Cu(II)/**L3B** complex is presented in Figure 1. Each of the three 2,2′-dipicolylamine moieties of **L3B** binds one Cu(II) ion. The angles for N(pyridine)−Cu−N(aliphatic) and N(pyridine)−Cu−N(pyridine) are 81.9−83.0° and 163.5−166.0°, respectively. The Cu−N(aliphatic) distance is 2.2026−2.034 Å, and the Cu−N(pyridine) distance is 1.967−1.982 Å. The structure reveals that each of the three 3,3′-dipicolylamine moieties complexes to one Cu(II) ion and they reside on one face of the triethylbenzene scaffold. However, each of the Cu(II) binding moieties points away from the interior of the cavity. This would indicate that there is little flexibility available in the receptor for the binding of ribonucleotide substrates.

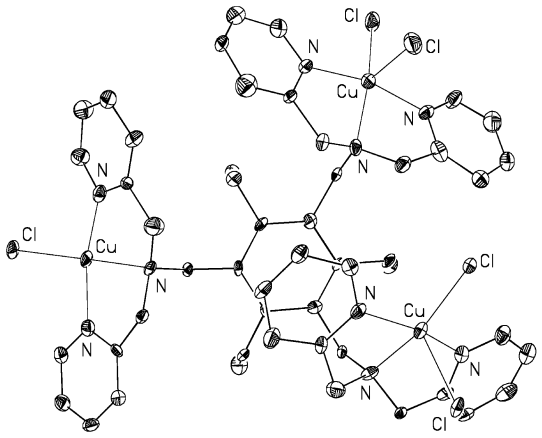


Figure 1. View of **L3B** showing the atom labeling scheme. Displacement ellipsoids are scaled to the 30% probability level. Hydrogen atoms have been removed for clarity.

Table 1. Pseudo-first-order Rate Constants (in 10^{-4} min^{-1}) for the Hydrolysis of (2′−5′)- and (3′−5′)-diribonucleotides by the Trinuclear Cu(II)/L3A Complex at pH 7.0 and 50°C[a,b]

ribonucleotide	2′–5′	3′–5′	linkage specificity[c]
UpU	28 (0.6)	0.5 (0.7)	56
GpG	0.3 (0.2)	0.3 (0.4)	1
ApA	0.5 (2.0)	24 (6.0)	0.021
CpC		7.0 (2.0)	

[a] $[Cu(II)]_0 = 6$ and $[\mathbf{L3A}]_0 = 2$ mM. [b]The values for the dinuclear Cu(II)/**L2** complex are presented in parentheses ($[Cu(II)]_0 = 6$ and $[\mathbf{L2}]_0 = 3$ mM). [c]The ratio of rate constant for the hydrolysis of (2′−5′)-diribonucleotide to the corresponding value for the (3′−5′)-isomer.

Hydrolysis of Diribonucleotides by Trinuclear Cu(II)/L3A Complex. Table 1 lists the results of diribonucleotide hydrolysis by the trinuclear Cu(II)/**L3A** complex at pH 7.0 (50 mM HEPES buffer) and 50 °C. This complex rapidly hydrolyzes Up(2′−5′)U to a 1:1 mixture of uridine and uridine monophosphate. Alternatively, Up(3′−5′)U is hydrolyzed at a significantly slower rate by this trinuclear Cu(II) complex.[13] The pseudo-first-order rate constant of the hydrolysis of the 2′−5′ isomer is 56-fold larger than that of the 3′−5′ isomer. It is interesting to note that the substrate specificity is reversed in the case of the ApA

(9) Vögtle, F.; Zuber, M.; Lichtenthaler, R.G. *Chem. Ber.* **1973**, *106*, 717.
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(11) The pK_a of the water bound to the Cu(II) ion in the bipyridine and terpyridine complexes are 7.0 and 8.08, respectively (ref 8a,d). The water bound to free Cu(II) ion has the pK_a 5.4−6.8: Burgess, J. *Metal Ions in Solution*; Horwood, Chichester, 1978.
(12) Walsdorf, C.; Park, S.; Kim, J.; Heo, J.; Park, K.; Oh, J.; Kim, K. *J. Chem. Soc., Dalton Trans.* **1999**, 923.
(13) The intrinsic activities of the 2′−5′ diribonucleotides and the 3′−5′ isomers are comparable with each other. Consistently, Up(2′−5′)U and Up(3′−5′)U are hydrolyzed at comparable rates under alkaline conditions and also in the presence of Mg(II), Mn(II), Co(II), Ni(II), and Zn(II): Kuusela, S.; Lönnberg, H. *J. Phys. Org. Chem.* **1993**, *6*, 347.

hydrolysis. Ap(3′−5′)A is hydrolyzed with a rate constant that is 48-fold larger than that of the Ap(2′−5′)A substrate. Therefore, the catalysis is selective with respect to both the type of phosphodiester linkage and the base sequence. Further, Gp-(3′−5′)G and Gp(2′−5′)G are not hydrolyzed to a significant extent, and the 2′−5′/3′−5′ specificity is negligible, yet Cp-(3′−5′)C is hydrolyzed at a reasonable rate. Each of the reactions described proceeds via an intramolecular attack by the 3′-OH (or 2′-OH) of the ribose. This is further confirmed by the fact that the 2′-deoxyribonucleotides are not hydrolyzed to a measurable extent by this complex. However, the intermediate 2′,3′-cyclic monophosphates do not accumulate, but are rapidly hydrolyzed to the final product ribonucleoside monophosphate.

For the purposes of comparison, the results with the dinuclear Cu(II) complex of *N*,*N*,*N*′,*N*′-tetra[(2-pyridyl)methyl]-1,3-bisaminoethylbenzene (**L2**) are also reported in Table 1. In the hydrolysis of Up(2′−5′)U, the trinuclear **L3A** complex is 47-fold more active than the dinuclear **L2** complex. For Ap(3′−5′)A hydrolysis, the **L3A** complex is 4-fold more active than that of the **L2** complex. Both the mononuclear Cu(II) complex of *N*,*N*-bis[(2-pyridyl)methyl]-(aminoethyl)benzene (**L1**) and Cu(II) ion are still less active than the dinuclear **L2** complex.[14,15] In the case of the other diribonucleotides, the differences in the activity between the trinuclear complex and the dinuclear complex are smaller. This indicates that the presence of the third Cu(II) ion in the trinuclear Cu(II)/**L3A** complex significantly accelerates the hydrolysis of Up(2′−5′)U and Ap(3′−5′)A.

Catalysis by Trinuclear Cu(II)/L3B Complex. In contrast to the efficient and substrate-specific catalyses by trinuclear Cu-(II)/**L3A** complex, the trinuclear Cu(II)/**L3B** complex demonstrates lower catalytic activity in the hydrolysis of the diribonucleotide substrates investigated. The pseudo-first-order rate constants are 0.1×10^{-4} min^{-1} for Up(2′−5′)U, 0.1×10^{-4} min^{-1} for Up(3′−5′)U, 0.02×10^{-4} min^{-1} for Ap(2′−5′)A, 0.1×10^{-4} min^{-1} for Ap(3′−5′)A, and 0.1×10^{-4} min^{-1} for Cp-(3′−5′)C. As observed in the catalysis by the **L3A** complex, Ap(3′−5′)A is more susceptible to hydrolysis than is Ap(2′−5′)A. However, no specificity was observed between Up(2′−5′)U and Up(3′−5′)U. The ligand **L3B** is similar in structure to **L3A**, the only difference being the restricted motions of the dipicolylamine moieties that result from the presence of the alternating ethyl groups on the benzene scaffold. Apparently, the molecular flexibility inherent in the **L3A** ligand is essential for the trinuclear Cu(II)/**L3A** complex to effect efficient and substrate-specific catalysis.

Kinetic Studies on the Catalysis by Trinuclear Cu(II)/L3A Complex. When the [Cu(II)] is kept constant and the $[Cu(II)]_0/[\mathbf{L3A}]_0$ ratio is varied, the rate of Up(2′−5′)U hydrolysis results in a maximum value at a ratio of 3 (open circles in Figure 2). A similar result is obtained for Ap(3′−5′)A hydrolysis (closed circles). At $[Cu(II)]_0/[\mathbf{L3A}]_0$ ratios greater than three, the trinuclear Cu(II) complex is quantitatively formed in proportion to the added ligand **L3A,** and the hydrolysis rate monotonically increases. On further addition of **L3A**, the fractions of mononuclear and dinuclear Cu(II) complexes gradually increase, diminishing the catalytic activity. These results confirm that the catalytic activity is primarily due to the Cu(II)/**L3A** complex. The contributions to the catalysis by the dinuclear Cu(II) complex, the mononuclear Cu(II) complex, and the Cu(II) ion are marginal.

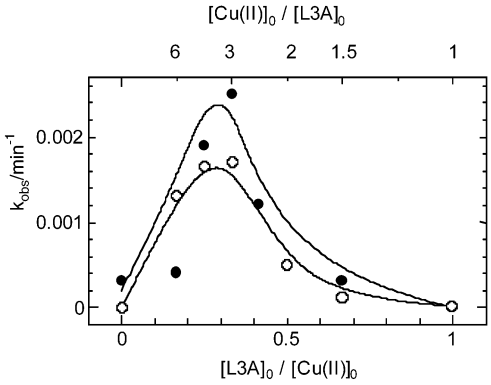

Figure 2. Plots of the hydrolysis rates of Up(2′−5′)U (open circles) and Ap(3′−5′)A (closed circles) vs the $[Cu(II)]_0/[\mathbf{L3A}]_0$ ratio at 50°C and pH 7.0: $[Cu(II)]_0$ is kept constant at 0.6 mM for Up(2′−5′)U and 6 mM for Ap(3′−5′)A.

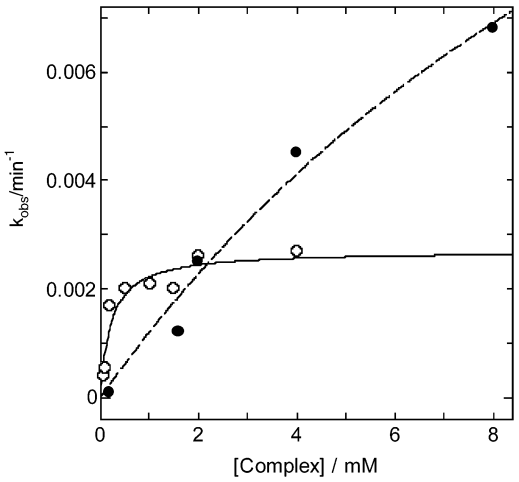

Figure 3. Dependencies of the hydrolysis rates of Up(2′−5′)U (open circles) and Ap(3′−5′)A (closed circles) on the concentration of the trinuclear Cu(II)/**L3A** complex ($[Cu(II)]_0$: $[\mathbf{L3A}]_0$ = 3:1) at 50°C and pH 7.0: The solid and the dotted lines are theoretical ones calculated by using the parameters in Table 2.

Table 2. Kinetic parameters for the trinuclear Cu(II)/**L3A** complex-induced hydrolysis of Up(2′−5′)U and Ap(3′−5′)A at pH 7.0 and 50°C[a]

substrate	k_{cat} (10^{-4} min^{-1})	K_m (mM)
Up(2′−5′)U	27	0.21
Ap(3′−5′)A	210	17

[a] Experimental error is estimated to be around 20%.

As shown in Figure 3, the rate of hydrolysis increases with increasing concentration of the Cu(II)/**L3A** complex with eventual saturation.[16] The values of k_{cat} and K_m, determined from Lineweaver−Burk plots, are presented in Table 2. Significantly, the K_m value for Up(2′−5′)U hydrolysis is much smaller than the corresponding value for Ap(3′−5′)A hydrolysis, but the k_{cat} is significantly smaller. Thus, the efficient hydrolysis of Up-(2′−5′)U by the trinuclear Cu(II)/**L3A** is primarily attributed

(14) The rate constants for the hydrolysis of Up(3′−5′)A and Ap(3′−5′)U by the Cu(II)/**L1** complex are 3.0×10^{-4} and 1.0×10^{-4} min^{-1}, respectively.
(15) Hydrolysis of bis(2,4-dinitrophenyl)phosphate by the Cu(II) complex of bis(2-pyridyl)methylamine was reported: Young, M. J.; Wahnon, D.; Hynes, R. C.; Chin, J. *J. Am. Chem. Soc.* **1995**, *117*, 9441.
(16) Here, the $[\mathbf{L3A}]_0/[Cu(II)]_0$ ratio is kept constant at 1/3. Note that the complex formation is almost quantitative, as described in the text.

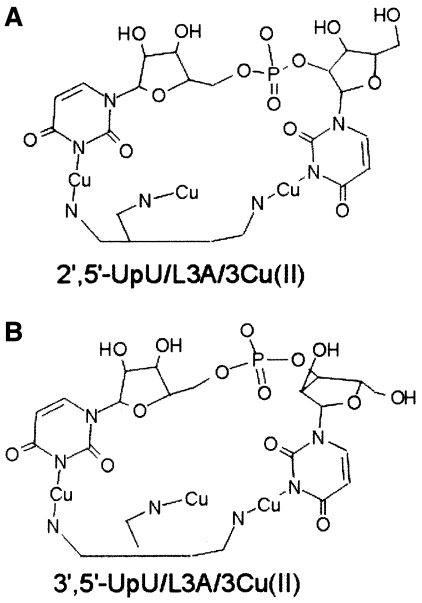

Figure 4. Schematic drawings of the adducts between the trinuclear Cu(II)/**L3A** complex and various dinucleotides: (A) Up(2′−5′)U and (B) Up(3′−5′)U.

to the strong binding of this substrate to this complex, whereas the large k_{cat} value is responsible for the rapid hydrolysis of Ap(3′−5′)A.

Possible Mechanisms for Substrate-Specific Hydrolysis. The hydrolysis of Up(2′−5′)U by the trinuclear Cu(II)/**L3A** complex is characterized by a very small K_m value (0.21 mM: **Table 2**). This value is far smaller than the value (47 mM) for the coordination of 4-nitrophenyl ethyl phosphate to the 2,2′-bipyridine/Cu(II) complex.[8a,17] Furthermore, it is even smaller than the value (0.63 mM)[18] for the coordination of HPO_4^{-2} to Cu(II) ion, although this dianionic ligand should be more favorable for the coordination than is UpU, from the viewpoint of electrostatic interactions. For the hydrolysis of Ap(3′−5′)A, however, substrate-binding is not promoted (K_m = 17 mM). These results indicate that the two uridine residues of UpU are coordinated to the Cu(II) ions in the **L3A** complex.[19] In fact, literature precedent substantiates coordination of uracil to Cu(II) ion at its N3 atom.[20]

The proposed structures of the adducts between UpU and the Cu(II)/**L3A** complex are schematically depicted in Figure 4. The third Cu(II) ion of this trinuclear complex is the catalytic center. In the adduct of Up(2′−5′)U (**A**), the scissile phosphodiester linkage is located nearer to this Cu(II) ion than in the adduct of Up(3′−5′)U (**B**), simply because the uracil is bound to the 1′-carbon atom of the ribose. Despite many rotatable bonds, these structures are rather rigid because of their ring-forms. The **L3A** ligand is flexible enough to allow the formation of this substrate-catalyst adduct and the conformational change required for the catalysis.[21] By contrast, the rigidity of the **L3B** ligand resulting from the alternating ethyl groups likely gives a less tightly held substrate-catalyst adduct and reduces the efficiency of hydrolysis for both Up(2′−5′)U and Up(3′−5′)U. The **L3B** complex is a poor catalyst for promoting the hydrolysis of all other diribonucleotides.

The dinuclear **L2** complex is less active in the hydrolysis of Up(2′−5′)U because only one Cu(II) ion is available for complex formation with the uracil moieties. For efficient transformation, at least one Cu(II) ion must bind the phosphodiester linkage to activate it. These arguments are supported based on the following experiments using Zn(II) ions which are known to strongly bind uracil.[22] In the hydrolysis of Up(2′−5′)U, the trinuclear Zn(II) complex of **L3A** is 14 times more active than that of the dinuclear Zn(II) complex of **L2**. For Ap(3′−5′)A hydrolysis, however, the trinuclear Zn(II) complex is almost as active as the dinuclear Zn(II) complex.

In the case of the hydrolysis of Ap(3′−5′)A by the trinuclear Cu(II)/**L3A** complex, the adenine moieties may hydrophobically stack with the aromatic groups in the **L3A** ligand, because direct coordination of adenine to Cu(II) ion is inefficient.[20] To maximize the overlap between these apolar moieties and stabilize the substrate-catalyst adduct, the trinuclear Cu(II) complex is pulled toward the phosphodiester linkage. This enables coordination of the third Cu(II) ion to the phosphodiester, activating it for hydrolysis. The hydrolysis of the (2′−5′) isomer is less efficient due to unfavorable steric factors.[23] The proposed importance of steric factors is supported by the fact that the trinuclear Cu(II)/**L3A** complex is less active than the dinuclear Cu(II)/**L2** complex only in this substrate (see Table 1). Consistently, both Gp(3′−5′)G and Gp(2′−5′)G are not hydrolyzed by this complex despite the fact [20] that guanine coordinates Cu(II) ions at N7. The coordination of the guanine moieties to two of the Cu(II) ions in the **L3A** complex orient the phosphodiester linkage away from the third Cu(II) ion, thereby impeding catalytic hydrolysis. Similar recognition of nucleic bases by dinuclear Cu(II) complexes was previously proposed for the hydrolysis of 2′,3′-cyclic monophosphates of ribonucleosides.[24] The base-selectivities were ascribed to the difference in the strength of hydrogen-bonding and/or stacking interaction between the complex and the substrate.

(17) The K_m for the coordination of bis(2,4-nitrophenyl)phosphate to the Cu(II) complex of a modified bipyridine-ligand is 13 mM in 19/1 ethanol/water mixture; Kövári, E.; Krämer, R. *J. Am. Chem. Soc.* **1996**, *118*, 12 704.

(18) Sigel, H.; Decker, K.; McCormick, D. B., *Biochim. Biophys. Acta*, **1967**, *89*, 1987.

(19) With regards to these results, the possibility that the third picolylamine-Cu moiety in the Cu(II)/L3A complex simply forces the other two moieties to the same side of the central benzene ring is unlikely. If this were the case, and only one of the three picolylamine-Cu moieties were binding UpU (another moiety must function as the catalyst), then the UpU binding by this complex could never be so unusually efficient.

(20) The binding constants for the coordination of uridine (N3) and guanosine (N7) are $10^{5.9}$ and $10^{5.58}$, respectively: *Biocoordination Chemistry: Coordination Equilibria in Biologically Active Systems*, Kálmán Burger, **1990**, Ellis Harwood: New York. The value for adenine is $10^{2.25}$ (the coordination site is unclear).

(21) This argument does not imply that the conformation in which all the three dipicolylamine-Cu moieties exist in the same plane with respect to the central benzene is the most stable one. Rather, the flexible nature of this complex allows it to take this conformation for the cooperative catalysis. According to the molecular modeling, the distance between the phosphorus atom and the third Cu(II) ion in 2′−5′ UpU is 8.2 Å, which is by 0.6 Å smaller than that in its 3′−5′ counterpart. For the chemical transformation in the catalysis, the system should show conformational change to place the third Cu(II) ion closer to the scissile phosphodiester linkage. In this calculation, the structure of the adduct between the trinuclear Cu complex and UpU was first optimized under the assumptions that (1) both the two uracil residues and the phosphate are coordinated to the three Cu(II) ions, and (2) the coordination modes of these Cu(II) complexes are square-planar. Then, the phosphate was removed from the corresponding Cu(II), to which a water molecule was bound instead. Finally, the whole system was optimized.

(22) The binding constant of uridine(at the N3 atom) to Zn(II) is $10^{3.57}$ (data from ref 20).

(23) According to the molecular modeling study, the distances between the P atom and the third Cu(II) ion are 4.6 Å for Ap(3′−5′)A and 5.8 Å for Ap(2′−5′)A, respectively.

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Conclusion

Trinuclear Cu(II) complex of **L3A** hydrolyzes 2′−5′ and 3′−5′ ribonucleotides with remarkable substrate specificity. Up(2′−5′)U is hydrolyzed more efficiently than is Up(3′−5′)U, but Ap(3′−5′)A is overwhelmingly preferred to Ap(2′−5′)A. Neither Gp(2′−5′)G nor Gp(3′−5′)G is hydrolyzed to any extent. This complex is, to the best of our knowledge, the first multinuclear metal complex that clearly distinguishes between 2′−5′ phosphodiester linkages and 3′−5′ phosphodiester linkages. The corresponding dinuclear Cu(II) complex of **L2** shows little substrate-specificity, indicating that three Cu(II) ions are necessary. The data reported herein demonstrates that the cooperation of several metal ions in multinuclear metal complexes can give rise to notable substrate-specificity in addition to large accelerations of hydrolysis. A design criteria for catalysis of RNA dinucleotide hydrolysis in a sequence dependent manner has emerged.

Experimental Section

Materials. Diribonucleotides were purchased from Seikagaku Kogyo. Highly purified water (the specific resistance > 18.3 ohm·cm) was sterilized at 120 °C immediately before use. Other chemicals were commercially obtained. Throughout the present study, great care was taken to avoid contamination by natural enzymes and other metal ions. The dinuclear ligand *N*,*N*,*N*′,*N*′,-tetra[(2-pyridyl)methyl]-1,3-bisaminoethylbenzene (**L2**) was synthesized and purified according to the literature method.[24]

Preparation of the Ligands L3A, L3B, and L2. (1) *N*,*N*,*N*′,*N*′,*N*″,*N*″-Hexa[(2-pyridyl)methyl]-1,3,5-tris(aminomethyl)benzene (L3A). The starting material 1,3,5-tris(bromomethyl)benzene was synthesized as follows.[9] Mesitylene (35 mL, 0.25 mol) and N-bromosuccinimide (1.42 g, 0.80 mol) were dissolved in 600 mL of carbon tetrachloride, and benzoyl peroxide (10 mg) was added with a bamboo spoon. After the solution was refluxed for 96 h under irradiation from a UV lamp, the precipitates were removed by suction filtration and the filtrate was evaporated. The solid material was dissolved in 200 mL of chloroform and washed with sodium bicarbonate solution (150 mL) and then with distilled water (450 mL). The organic layer was dried with sodium sulfate and evaporated. The product was dissolved in petroleum ether, filtrated, and recrystallized from chloroform/petroleum ether to needle-shaped crystals (2.5 g).

To a stirred solution of 1,3,5-tris(bromomethyl)benzene (100 mg, 0.28 mol) in acetonitrile (18 mL), was added 2,2′-dipicolylamine (167 mg, 0.84 mmol) and *N*,*N*-diisopropylethylamine (116 mg, 0.90 mmol). The resulting solution was stirred at room temperature overnight, and the solvent was removed to yield a brown product which was purified by silica gel chromatography (acetonitrile/triethylamine = 9/1 (v/v)). ^{1}H NMR (500 MHz, $CDCl_3$ / TMS) δ 8.50 (d, J = 5.0 Hz, 6H, Py-H_6), 7.54−7.59 (m, 12H, Py-H_3, H_4), 7.36 (3H, Bz-*H*), 7.11 (m, 6H, Py-H_5), 3.81 (12H, NCH_2Py), 3.68 (6H, NCH_2Bz); ^{13}C NMR (125.6 MHz, $CDCl_3$/TMS) δ 159.9, 149.0, 139.2, 136.3, 128.0, 122.7, 121.9, 60.2, 58.7.

(2) *N*,*N*,*N*′,*N*′,*N*″,*N*″-Hexa[(2-pyridyl)methyl]-1,3,5-tris(aminomethyl)-2,4,6-triethylbenzene (L3B). 1,3,5-Tris(chloromethyl)-2,4,6-triethylbenzene [10] (640 mg, 2.1 mmol) was dissolved in acetonitrile (25 mL), and to this solution 3,3′-dipicolylamine (1.65 g, 8.3 mmol) and then potassium carbonate (862 mg, 6.3 mmol) were added. The reaction mixture was refluxed for 72 h and filtered through a bed of diatomaceous earth using dichloromethane. By removing the solvent, a brown solid was obtained. The crude mixture was triturated with cold ethyl acetate and decanted. The light brown solid was recrystallized using ethyl acetate/hexane to yield white crystals (148 mg, 60% yield): ^{1}H NMR (300 MHz, CD_3OD/TMS) δ 8.33 (d, J = 4.8, 6H), 7.51 (dt, J = 1.5, J = 7.8, 6H), 7.22 (d, J = 8.1, 6H), 7.11 (m, 6H), 3.65 (s, 12H), 3.63 (s, 6H). 2.92 (q, J = 7.5, 6H) 0.65 (t, J = 7.2, 9H); ^{13}C NMR (CD_3OD, 300 MHz) δ 160.5, 149.1, 146.6, 138.4, 125.1, 123.6, 60.5, 51.9, 23.2, 16.0; HRMS (CI, *m*/*z*) calcd for $C_{51}H_{57}N_9$ 796.48, found 796.48.

Hydrolysis of Diribonucleotides. The reaction mixtures were prepared by adding an acetonitrile solution of the ligand to 50 mM HEPES buffer containing $Cu(ClO_4)_2$. The pH was adjusted to 7.0 using aliquots of NaOH. A stock solution of the substrate in water (10 mM) was added, and the reaction was carried out at 50 °C. The initial concentration of the substrate was 0.1 mM. At appropriate intervals, a small portion of the reaction mixture was taken and combined with an aqueous EDTA (100 mM) solution. The specimen was treated with a pretreatment filter (Tosoh, W-3−2) and analyzed by reverse-phase HPLC (Merck LiChrospher RP-18(e) ODS column; water/acetonitrile = 92/8 (v/v)). The HPLC peaks were assigned by co-injection with

Table 3. Crystal Data and Structure Refinement for L3B

empirical formula	C113 H158 Cl12 Cu6 N18 O11	
formula weight	2751.22	
temperature	153(2) K	
wavelength	0.71073 Å	
crystal system	monoclinic	
space group	P21	
unit cell dimensions	a = 12.1429(3) Å	α= 90°.
	b = 33.2454(8) Å	β= 94.494(1)°.
	c = 15.7008(4) Å	γ = 90°.
volume	6318.9(3) Å^3	
Z	2	
density (calculated)	1.446 Mg/m^3	
absorption coefficient	1.308 mm^{-1}	
F(000)	2856	
crystal size	0.22 × 0.10 × 0.04 mm	
theta range for data collection	2.97 to 26.55°.	
index ranges	−15<=h<=14, −40<=k<=33, −18<=l<=18	
reflections collected	18056	
independent reflections	18056	
refinement method	Full-matrix-block least-squares on F^2	
data/restraints/parameters	18056/967/1455	
goodness-of-fit on F^2	1.867	
final *R* indices [I>2sigma(I)]	*R*1 = 0.116, *wR*2 = 0.164	
R indices (all data)	*R*1 = 0.195, *wR*2 = 0.180	
absolute structure parameter	0.11(2)	
largest diff. peak and hole	1.024 and −0.933 e.Å^{-3}	

authentic samples. All reactions satisfactorily showed pseudo-first-order kinetics, and the pH change during the reactions was less than 0.1 pH unit.

UV−Visible Absorption Spectra and Potentiometric Titration. The spectra were measured on a JASCO U-520 spectrometer equipped with a temperature-controller. The cell path length was 1.0 cm. All of the potentiometric titration for the Cu(II) complexes was carried out at 25 °C.

X-ray Crystallography. The ligand **L3B** and 3 equiv of $CuCl_2$ were dissolved in methanol and allowed to evaporate slowly at 25 °C to yield a blue crystal.

X-ray Experimental for $[C_{51}H_{57}N_9Cu_3Cl_3]_2-2Cl^- - 11CH_3OH$: Crystals grew as pale blue needles by slow evaporation from methanol. Data were collected at 153 K on a Nonius Kappa CCD diffractometer using a graphite monochromator with MoKα radiation ($\lambda = 0.71073$ Å). A total of 594 frames of data were collected using ω-scans with a scan range of 0.5° and a counting time of 98 s per frame. Details of crystal data and structure refinement are listed in Table 3. Data reduction were performed using DENZO−SMN.[26] The structure was solved by direct methods using SIR92[27] and refined by full-matrix least-squares on F^2 with anisotropic displacement parameters for the non-H atoms using SHELXL-97.[28] The hydrogen atoms on carbon were calculated in ideal positions with isotropic displacement parameters set to 1.2 × Ueq of the attached atom (1.5 × Ueq for methyl hydrogen atoms). Hydrogen atom positions in the vicinity of the oxygen atoms of the methanol molecules were not located in a ΔF map. As a result, these hydrogen atoms were not included in the refinement model. The absolute structure was determined by the method of Flack.[29] The Flack x parameter refined to 0.11(2). The function, $\Sigma w(|F_o|^2 - |F_c|^2)^2$, was minimized, where $w = 1/[(\sigma(F_o))^2 + (0.02*P)^2]$ and $P = (|F_o|^2 + 2|F_c|^2)/3$. $R_w(F^2)$ refined to 0.180, with $R(F)$ equal to 0.116 and a goodness of fit, S, = 1.87. Definitions used for calculating $R(F)$,- $R_w(F^2)$ and the goodness of fit, S, are given below.[30]

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Supporting Information Available: X-ray crystallographic data (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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