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Article

Structure–Activity Studies on the Cleavage of an RNA Analogue by a Potent Dinuclear Metal Ion Catalyst: Effect of Changing the Metal Ion

Olga Iranzo, John P. Richard,* and Janet R. Morrow*

Department of Chemistry, University at Buffalo, State University of New York, Buffalo, New York 14260

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Dinuclear Cd(II), Cu(II), and Zn(II) complexes of **L2OH** (**L2OH** = 1,3-bis(1,4,7-triazacyclonon-1-yl)-2-hydroxypropane) are compared as catalysts for cleavage of the RNA analogue **HpPNP** (**HpPNP** = 2-hydroxypropyl 4-nitrophenyl phosphate) at 25 °C, $I = 0.10$ M ($NaNO_3$). Zn(II) and Cu(II) readily form dinuclear complexes at millimolar concentrations and a 2:1 ratio of metal ion to **L2OH** at neutral pH. The dinuclear Zn_2**(L2O)** and Cu_2**(L2O)** complexes have a bridging alkoxide group that brings together the two cations in close proximity to facilitate cooperative catalysis. Under similar conditions, the dinuclear complex of Cd(II) is a minor species in solution; only at high pH values (pH 10.4) does the Cd_2**(L2O)** complex become the predominant species in solution. Analysis of the second-order rate constants for cleavage of **HpPNP** by Zn_2**(L2O)** is straightforward because a linear dependence of pseudo-first-order rate constant on dinuclear complex is observed over a wide pH range. In contrast, plots of pseudo-first-order rate constants for cleavage of **HpPNP** by solutions containing a 2:1 ratio of Cd(II) to **L2OH** as a function of increasing **L2OH** are curved, and second-order rate constants are obtained by fitting the kinetic data to an equation for the formation of the dinuclear Cd(II) complex as a function of pH and [**L2OH**]. Second-order rate constants for cleavage of **HpPNP** by these dinuclear complexes at pH 9.3 and 25 °C vary by 3 orders of magnitude in the order Cd_2**(L2O)** ($2.8\ M^{-1}\ s^{-1}$) > Zn_2**(L2O)** ($0.68\ M^{-1}\ s^{-1}$) > Cu_2**(L2O)** ($0.0041\ M^{-1}\ s^{-1}$). The relative reactivity of these complexes is discussed in terms of the different geometric preferences and Lewis acidity of the dinuclear Zn(II), Cu(II), and Cd(II) complexes, giving insight into the importance of these catalyst properties in the cleavage of phosphate diesters resembling RNA.

Introduction

The dinuclear complex Zn_2**(L2O)** represents a very successful achievement in the design of small molecular weight metal ion complexes with unusual catalytic activity.[1,2] We have shown that this catalyst at pH 7.0 provides 9.5 kcal/mol stabilization of the transition state for cleavage of the small molecular weight substrate **HpPNP** and a somewhat smaller 7.1 kcal/mol stabilization of the transition state for cleavage of the nucleoside substrate **UpPNP**.[3] Both substrates undergo cleavage by transesterification at the phosphate diester to form a cyclic phosphate product. The critical element in the design of Zn_2**(L2O)** is the inclusion of the 2-hydroxyl functional group at the propyl bridge in the free ligand **L2OH**. This hydroxyl group ionizes with an extraordinarily low pK_a of < 6.0 to form **L2O** and functions as an electrostatic “shield” which allows the Zn(II) ions to be drawn relatively close together to form a cationic catalytic core. We have found that many dinuclear metal ion catalysts show only marginally greater than twice the catalytic activity of the appropriate related mononuclear complexes.[2] Therefore, the simple tethering of these complexes is not sufficient to observe efficient catalysis by two metal ion centers. By comparison, the catalytic activity of Zn_2**(L2O)** is 120-fold larger than the mononuclear complex so that the Zn(II) cations at the core of this complex show true cooperativity in stabilizing the transition state for cleavage of phosphodiester substrates.

Research to design effective catalysts of the cleavage of RNA is largely with the goal of realizing the potential of

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* To whom correspondence should be addressed. E-mail: jmorrow@buffalo.edu (J.R.M.); jrichard@buffalo.edu (J.P.R.).

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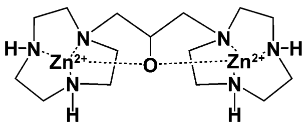
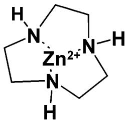
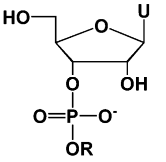


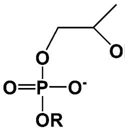
Zn_2**(L2O)**



Zn(L1)



UpPNP



HpPNP

R=C_6H_4-4-NO_2

the many useful medicinal and clinical applications for these complexes.[4-7] There is the broader question of the origin of the catalytic rate acceleration of small molecular weight catalysts such as Zn_2**(L2O)** and larger molecular weight protein and RNA catalysts. Experiments to resolve these questions are of considerable intellectual interest and likewise have the potential to suggest new and better strategies for the design of effective catalytic agents.

Structure−activity studies have long provided useful insight into the mechanism of large and small molecular weight catalysts. We report here the results of a detailed study of the effect on catalytic activity of changing the metal cation in M_2**(L2O)** complexes. The observation of a very low catalytic activity for Cu_2**(L2O)** shows that catalysis is highly dependent on the coordination number of the metal cation. The Cd(II) and Zn(II) cations are isoelectronic, and Zn_2**(L2O)** and Cd_2**(L2O)** might therefore have been predicted to show similar catalytic activity toward phosphodiester cleavage. In one sense this prediction is confirmed by the experimental results reported here. On the other hand, the subtle differences in the catalytic properties of Zn_2**(L2O)** and Cd_2**(L2O)** are significant with respect to the interesting insight that they provide into the structure and catalytic activity of the cationic core of these catalysts.

Experimental Section

Materials. All reagents and solvents were of reagent grade and were used without further purification, unless otherwise noted. All aqueous solutions were prepared using Millipore MILLI-Q purified water. 2-Morpholinoethanesulfonic acid (MES), *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (HEPES), *N*-(2-hydroxyethyl)piperazine-*N*′-(3-propanesulfonic acid) (EPPS), 2-(cyclohexylamino)ethanesulfonic acid (CHES), and 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS) were purchased from Sigma-Aldrich. The barium salt of 2-hydroxypropyl 4-nitrophenyl phosphate (**HpPNP**)[8] and **L2OH**[2] were synthesized according to published procedures.

An aqueous stock solution (40.0 mM) of the ligand **L2OH** was prepared, and the concentration was determined by ^{1}H NMR using *p*-toluenesulfonic acid as an internal standard. Solutions of Cu(NO_3)$_2$ (Mallinckrodt) and Cd(NO_3)$_2$ (Fisher) were standardized with EDTA using Fast Sulphon Black and Xylenol Orange as indicators, respectively. Stock solutions (5.00 mM) of the Cu(II) and Cd(II) complexes of **L2OH** for kinetic experiments were prepared in water by mixing the corresponding metal salt with the HCl salt of **L2OH** in 1:1.1 (mononuclear system) and 2:1.1 (dinuclear system) molar ratios and adjusting the pH to 6.5−7.0 with NaOH.

An Orion Research Digital Ionalyzer/501 and an Orion Research Ross Combination pH Electrode 8115BN were used for all pH measurements. An UVIKON-XL spectrophotometer by Bio-Tek instruments equipped with a thermostated multicell transfer compartment was used for all kinetic measurements. ^{1}H NMR spectra were recorded on a Varian Inova 500 spectrometer. ^{31}P NMR spectra were recorded at 169 MHz on an Inova Varian 400 spectrometer. ^{31}P chemical shifts are reported as parts per million (ppm) downfield from 85% H_3PO_4.

Electrospray Ionization Mass Spectrometry (ESI-MS). A ThermoFinnigan LCQ Advantage electrospray mass spectrometer was used for ESI-MS analysis with 100% water at 180 °C. The sample was run by direct infusion from a Cole Parmer 74900 series syringe pump at 10 μL min^{-1} in the positive-ion mode. Stock solutions (1.0 mM) of the Cu(II) and Cd(II) complexes of **L2OH** were prepared in water by mixing the corresponding metal salt with the HCl salt of **L2OH** in 1:1 (mononuclear system) and 2:1 (dinuclear system) molar ratios and adjusting the pH to 7.0 with an aqueous solution of ammonia. Samples were diluted with water to 50.0 μM, and the pH was adjusted to 7.4 for Cd(II) and 7.3 for Cu(II) with an aqueous solution of ammonia.

ESI-MS (H_2O) [*m*/*z* (relative intensity)]: [Cd(**L2OH**)]$^{+2}$, 212.3 (40%), 212.8 (49%), 213.3 (88%), 213.8 (55%), 214.3 (100%), 214.8 (19%), 215.3 (26%).

ESI-MS (H_2O) [*m*/*z* (relative intensity)]: [Cd(**L2O**)]$^+$, 423.1 (42%), 424.1 (50.5%), 425.1 (86.5%), 426.1 (55%), 427.1 (100%), 428.1 (18%), 429.1 (27%).

ESI-MS (H_2O) [*m*/*z* (relative intensity)]: [Cd(**L2OH**)Cl]$^+$, 459.1 (34.5%), 460.1 (40%), 461.1 (78.5%), 462.1 (54.5%), 463.1 (100%), 464.1 (29.5%), 465.1 (46.5%).

ESI-MS (H_2O) [*m*/*z* (relative intensity)]: [Cd_2(**L2O**)(Cl)$_2$]$^+$, 602.9 (13%), 604.0 (15%), 605.0 (39.5%), 606.0 (37%), 606.9 (77.5%), 607.9 (73.5%), 608.9 (100%), 609.9 (72%), 610.9 (98%), 611.9 (42.5%), 612.9 (54.5%), 613.9 (19.5%), 614.9 (18%).

ESI-MS (H_2O) [*m*/*z* (relative intensity)]: [Cu_2(**L2O**)(Cl)$_2$]$^+$, 509.1 (65%), 510.1 (12.5%), 511.1 (100%), 512.0 (19.5%), 513.0 (59%), 514.0 (11%), 515.0 (15.5%), 516.0 (4%).

NMR Spectroscopic Measurements. The formation and stoichiometry of the Cd(II) complexes of **L2OH** in D_2O was studied by ^{1}H NMR. ^{1}H NMR spectra were recorded for solutions that contained 1.0 mM **L2OH** and concentrations of Cd(NO_3)$_2$ (I = 0.10 M, $NaNO_3$) that increased from 0 to 2.5 mM. The solution pD was calculated by adding 0.4 to the reading from the pH meter.[9] ^{31}P NMR showed that metal ion complex-catalyzed cleavage of **HpPNP** gave the cyclic phosphate diester as the sole phosphorus containing product.

Kinetics of Transesterification of HpPNP (2-Hydroxypropyl 4-Nitrophenyl Phosphate). Metal ion complex-catalyed cleavage of **HpPNP** (0.02 mM) at 25 °C in 20.0 mM buffer and at I = 0.10 M ($NaNO_3$) was monitored using procedures described in earlier

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work.[1] The reaction pH was determined at the end of each spectrophotometric experiment and found to be within 0.02 units of the initial value.

The relatively fast cleavage of **HpPNP** catalyzed by Cd(II) complexes of **L2OH** was generally monitored for 3 half-times, and pseudo-first-order rate constants k_{obsd} were determined as the slopes of semilogarithmic plots of reaction progress against time. The slower cleavage of **HpPNP** catalyzed by Cu(II) complexes of **L2OH** was monitored for the disappearance of the first 5−10% of the substrate, and the reaction end point was obtained by heating the reaction to 60 °C. Values of k_{obsd} (s^{-1}) were determined as $k_{obsd} = v_i/[S]_o$, where v_i is the initial reaction velocity and $[S]_o$ is the initial substrate concentration. The second-order rate constant for **HpPNP** cleavage by Cu_2**(L2O)** was determined as the slope of linear plots of k_{obsd} against the corresponding **L2OH** concentration (at pH 8.00). Second-order rate constants for Cd_2**(L2O)** were determined by fitting the data to eq 1 (see Discussion). Rate constants were reproducible to ±6%. Background cleavage rate constants[1] in the absence of added catalyst were subtracted from the relatively small pseudo-first-order rate constants for cleavage of **HpPNP** by free Cd(II) and solutions containing 1:1 ratios of Cd(II) to **L2OH** at pH 8.00, 9.30, and 10.0 and by Cu_2**(L2O)** at pH 7.00 and 9.30.

Results

M_2**(L2O)** is used to designate all dinuclear metal ion complex species present at a particular pH in solution. Specific species with hydroxide or water ligands will be indicated as needed.

The extent of association of Cd(II) to **L2OH** was examined by using mass spectrometry and ^{1}H NMR spectroscopy. Only the ion for the mononuclear complex of Cd(II) and **L2OH** was observed by ESI-MS analysis of aqueous solutions at pH 7.4 that contained a 1:1 ratio of 0.05 mM $Cd(NO_3)_2$ and **L2OH** (see Experimental Section). Analysis of solutions that contained a 2:1 ratio of $Cd(NO_3)_2$ and **L2OH** under similar conditions showed dominant peaks for the mononuclear complex and a minor peak for the dinuclear species. These results provide evidence that a single Cd(II) binds very tightly to **L2OH** and that the formation of the dinuclear complex is incomplete under these reaction conditions.

The ^{1}H NMR spectral data for **L2OH** in solutions that contained Cd(II) show a broad similarity to those reported for the complex between **L2OH** and Zn(II)[2] (Supporting Information Figures S1 and S2), but there are significant differences which provide evidence that the second Cd(II) binds with a weaker affinity to **L2OH** than the second Zn(II). At pD 8.1 and a 1:1 ratio of 1.00 mM Cd(II) to **L2OH**, the ^{1}H NMR spectrum is relatively simple with a multiplet at 3.9 ppm for the proton attached to the bridging alcohol group. This is consistent with the formation of a single type of mononuclear macrocyclic complex of relatively high symmetry (Figure S1, $r = 1.0$). While the structure of this complex is not certain, these data are most consistent with the Cd(II) ion being bound to both **L1** units to form a mononuclear complex with a sandwich structure and a free alcohol group. This has been reported for similar ligands containing **L1** and a three carbon linker[10] and for the analogous complex Zn**(L2OH)**.[2]

At pH 8.1 and a 2:1 ratio of $Cd(NO_3)_2$ to **L2OH** (1.00 mM), new resonances appear for the α-hydroxy proton that are shifted upfield with respect to the corresponding signals for the free ligand. This change parallels that observed for formation of Zn_2**(L2O)**,[2] where binding of the second metal cation is accompanied by loss of the alcohol proton and thus provides strong evidence for the formation of Cd_2**(L2O)** with a structure similar to that for Zn_2**(L2O)**. Resonances for the mononuclear complex continue to be observed (Figure S1, $r \geq 2.0$). By contrast, the formation of Zn_2**(L2O)** at similar pD and metal ion concentration is quantitative.[1,2] The proportion of dinuclear Cd(II) complex increases as the pD is raised (Figure S2), and at pD 10.4 the dinuclear complex is the major species in a solution that contains a 2:1 ratio of $Cd(NO_3)_2$ to **L2OH** (1.00 mM).

Pseudo-first-order rate constants (k_{obsd}) for cleavage of **HpPNP** at pH 8.0, 25 °C, and $I = 0.10$ M ($NaNO_3$) in solutions that contain 0.200 mM $Cd(NO_3)_2$ or for reactions under the same conditions but in the presence of a 1:1 ratio of $Cd(NO_3)_2$ to **L2OH** are more than 100-fold smaller than those determined for reactions using a 2:1 ratio of $Cd(NO_3)_2$ to **L2OH** (Supporting Information Table S1). Similarly, the values of k_{obsd} determined for reactions at pH 9.3−10 in solutions that contain free Cd(II) or a 1:1 ratio of Cd(II) to **L2OH** are more than 10 times smaller than the values determined for the corresponding reactions in solutions that contain a 2:1 ratio of $Cd(NO_3)_2$ to **L2OH**. These results provide strong evidence that the dinuclear complex Cd_2**(L2O)** is the most active form of the catalyst under these reaction conditions.

Figure 1 shows the increase in ($k_{obsd} - k_0$) with increasing concentrations of **L2OH** for the cleavage of **HpPNP** at a constant 2:1 ratio of $Cd(NO_3)_2$ to **L2OH** for reactions at 25 °C and $I = 0.10$ M ($NaNO_3$), where k_{obsd} is the observed first-order rate constant for the cleavage reaction and k_0 is the first-order rate constant for the reaction in the absence of catalyst at the same pH. There is severe upward curvature in the plots of data for experiments at low pH (see Figure 1 inset). This curvature decreases as the pH is increased, and at the highest pH of 10.5 the plot is effectively linear.

The following observations show that **L2OH** binds two Cu(II) ions under the conditions of our experiments and are consistent with earlier solution and solid-state studies which showed that two Cu(II) ions bind to **L2OH** to form Cu_2**(L2O)**.[11] (i) The addition of 2 equiv of $Cu(NO_3)_2$ to buffered solutions containing 0.500 mM **L2OH** gave clear deep blue solutions with no evidence of precipitation over the pH range 7.0−9.3. Under similar conditions in the absence of **L2OH** ligand, complexes of Cu(II) hydroxide precipitate. (ii) Analysis by electrospray ionization mass spectrometry (ESI-MS) of solutions that contained a 2:1 ratio of $Cu(NO_3)_2$ to **L2OH** at pH 7.3 showed the major peak to be that of the parent ion of the dinuclear complex $[Cu_2(L2O)(Cl)_2]^+$ and

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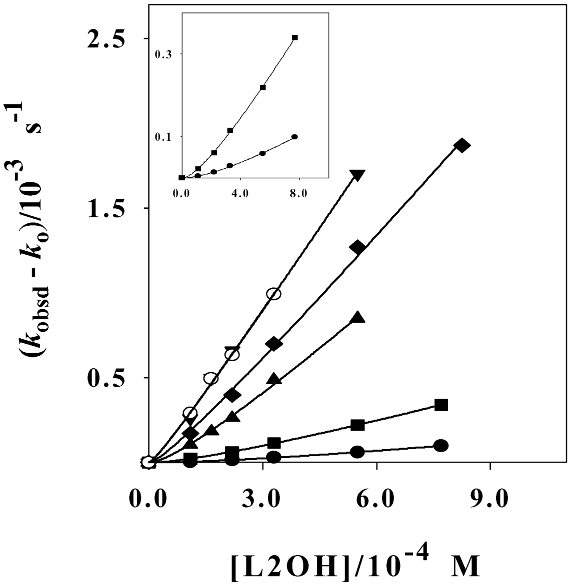

Figure 1. Pseudo-first-order rate constants for cleavage of **HpPNP** catalyzed by solutions containing a 2:1 ratio of Cd(II) to **L2OH** at 25 °C and $I = 0.10$ M ($NaNO_3$) as a function of **L2OH** at different pH values: (●) 8.02; (■) 8.43; (▲) 8.93; (◆) 9.31; (▼) 10.00; (○) 10.52. The solid lines show the fit of the data to eq 1. Data at pH 8.02 and 8.43 with an expanded *y*-axis are shown in the inset to demonstrate the curvature observed in these plots.

no peaks for a mononuclear complex (see Experimental Section). (iii) The observed first-order rate constants for cleavage of **HpPNP** are directly proportional to [**L2OH**] for experiments in which [Cu(II)] and [**L2OH**] are increased together at a constant ratio of 2 to 1 (Figure S3).

The second-order rate constant for the cleavage of **HpPNP** at pH 8.0, 25 °C, and $I = 0.10$ M ($NaNO_3$) by the dinuclear Cu(II) complex is $1.8 \times 10^{-3}\ M^{-1}\ s^{-1}$ (Figure S3). Second-order rate constants, calculated from data for reaction at a single concentration of Cu_2**(L2O)** (0.500 mM), at pH 7.0 and 9.3 are 2.0×10^{-3} and $4.1 \times 10^{-3}\ M^{-1}\ s^{-1}$, respectively. We assume that these are second-order rate constants for the cleavage reaction catalyzed by Cu_2**(L2O)** because this is the dominant metal ion species under these reaction conditions (see above). However, we are not able to rigorously exclude the possibility that a fraction of this reaction is catalyzed by free Cu(II) because free Cu(II) is a very good catalyst for **HpPNP** cleavage[12] and a very low concentration of Cu(II) might contribute to the low rate constants observed here. In this case, the second-order rate constants reported here would serve as upper limits for the rate constant for the reaction catalyzed by Cu_2**(L2O)**.

Discussion

We report here the results of a qualitative physical characterization of Cd(II) and Cu(II) complexes of **L2OH** by ^{1}H NMR and mass spectrometric methods. Mass spectrometric data define the dominant form of the Cu(II) and Cd(II) complexes at neutral pH. In addition, ^{1}H NMR spectroscopy is used to monitor the change in the dominant form of the Cd(II) **L2OH** complex over the pD range 8.1−10.4. We are unable to obtain equilibrium constants for formation of metal ion complexes of **L2OH** by using these spectrometric methods. However, the qualitative data from these experiments are critical to the development of the model used to fit kinetic data for **HpPNP** cleavage catalyzed by dinuclear **L2OH** complexes of Cd(II) and Cu(II). These fits of kinetic data provide values of equilibrium constants for formation of the dinuclear Cd(II) complex of **L2OH** that could not be obtained by spectrometric methods.

Spectroscopic Analysis. (a) Cd(II) Complex. L2OH shows a high affinity for Zn(II), and the dinuclear complex of this cation (Zn_2**(L2O)**) can be assembled quantitatively by mixing 2 equiv of Zn(II) with 1 equiv of **L2OH**.[1,2] In contrast, the dinuclear Cd(II) complex (Cd_2**(L2O)**) only becomes the dominant species in solutions that contain a 2:1 ratio of Cd(II) to **L2OH** at pH > 10.0. Both ^{1}H NMR and mass spectrometric analyses show that the dominant ligand species in water (pD = 8.1 and pH = 7.4, respectively) is the mononuclear Cd(II) complex. ^{1}H NMR analysis at intermediate pD ranging from pD = 8.1 to 10.4 shows that there is a progressive increase in the concentration of the dinuclear Cd(II) complex relative to the mononuclear complex. Kinetic data for metal ion complex-catalyzed cleavage of **HpPNP** discussed below provide additional evidence that the unreactive mononuclear Cd(II) **L2OH** complex predominates at pH 8.0 and undergoes quantitative conversion to the dinuclear complex Cd_2**(L2O)** at pH > 10.0. The Cd_2**(L2O)** complex, once formed, shows an extraordinary activity in catalyzing **HpPNP** cleavage.

(b) Cu(II) Complex. The limited data for the Cu(II) complex of **L2OH** are consistent with the formation of the dinuclear complex Cu_2(**L2O**) at pH 7.0−9.3. We have not carried out a thorough physical or kinetic characterization of this dinuclear complex, because our primary interest is in understanding the relationship between the structure and activity of *reactive* catalysts of phosphodiester cleavage, and only a very low catalytic activity is observed for the dinuclear Cu(II) complex of **L2OH**.

Kinetic Analyses. (a) Cd(II) Complex. The analysis of the kinetic data for catalysis of **HpPNP** cleavage by Zn_2**(L2O)** is straightforward. Plots of k_{obsd} against [Zn_2**(L2O)**] are linear, where k_{obsd} is the observed first-order rate constant for the cleavage reaction. The slopes of these plots are equal to the apparent second-order rate constant $(k_{Zn})_{app}$ for the Zn(II) complex-catalyzed reaction.[1] By comparison, plots of $k_{obsd} - k_0$ for the cleavage of **HpPNP** against [**L2OH**] (Figure 1) show pronounced upward curvature for an experiment in which the concentration of **L2OH** and Cd(II) are increased at a constant 2:1 ratio of Cd(II) to **L2OH**.

The curved plots from Figure 1 have been rationalized by a model where the mononuclear complex undergoes conversion to the dinuclear complex, as was shown by ^{1}H NMR to occur as the pD was increased from 8.1 to 10.4, and only the dinuclear complex Cd_2(**L2O**) has a significant activity toward cleavage of **HpPNP**. Consider the tangential lines through the curved plots shown in Figure 1. The slopes of these lines are *apparent* second-order rate constants for

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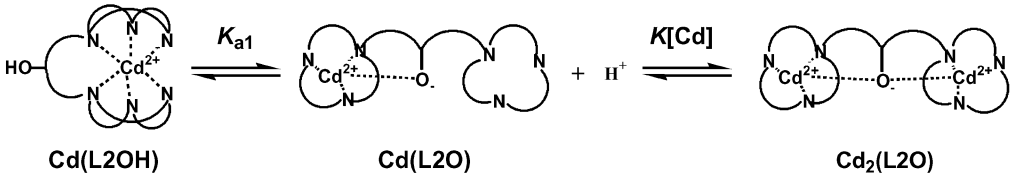
Scheme 2



catalysis of **HpPNP** cleavage by the dinuclear Cd(II) complex under the given reaction conditions. These slopes increase with increasing [**L2OH**] (at a 2:1 Cd(II) to **L2OH**) to a constant value at high [**L2OH**] where mass action has completely converted the mononuclear complex to the dinuclear complex. The limiting slope at high [**L2OH**] corresponds to the observed second-order rate constant for catalysis of **HpPNP** cleavage by Cd$_2$**(L2O)** and has the same significance as the second-order rate constants reported for catalysis of **HpPNP** cleavage by Zn$_2$**(L2O)**. In other words, the difference in the shape of the plots observed for catalysis by Zn(II) (linear) and by Cd(II) complexes (concave up) is due to the smaller extent of formation of the dinuclear Cd(II) complex and the requirement of higher concentrations of Cd(II) and **L2OH** to obtain complete conversion to the catalytically active dinuclear complex.

The experimental data from Figure 1 were fit to eq 1, which was derived for Scheme 1 with the assumptions that all **L2OH** has bound at least one Cd(II) ion and that the concentration of free Cd(II) is equal to the concentration of mononuclear complex (Cd**(L2OH)**$_T$). Note: (1) Cd**(L2OH)**$_T$ and Cd$_2$**(L2O)**$_T$ in Scheme 1 include a total of four different ionic forms of the mono- and dinuclear complexes. Cd**(L2OH)**$_T$ = Cd**(L2OH)** + Cd**(L2O)** includes two forms of the mononuclear complex that show a low (Cd**(L2OH)**) and a high (Cd**(L2O)**) affinity for binding of a second Cd(II) (see drawings in Scheme 2), and Cd$_2$**(L2O)**$_T$ = Cd$_2$**(L2O)**(OH$_2$) + Cd$_2$**(L2O)**(OH) includes two forms of the dinuclear complex that are catalytically inactive (Cd$_2$**(L2O)**(OH$_2$)) and active (Cd$_2$**(L2O)**(OH)) (see drawings in Scheme 3). (2) Equation 1 predicts a limiting slope of $(k_{Cd})_{app}$ at high [**L2OH**] and 2 equiv of Cd(II), where [**L2OH**] ≫ $[1/(2K_{app}) - ([\mathbf{L2OH}]/K_{app})^{1/2}]$.

Scheme 1

$$\mathrm{Cd}(\mathbf{L2OH})_T + \mathrm{Cd} \xrightleftharpoons{K_{app}} \mathrm{Cd}_2(\mathbf{L2O})_T \xrightarrow{(k_{Cd})_{app}[\mathrm{HpPNP}]} \text{products}$$

$$k_{obs} - k_0 = (k_{Cd})_{app}\left([\mathbf{L2OH}] + \left(\frac{1}{2K_{app}}\right) - \left(\frac{\sqrt{1 + 4[\mathbf{L2OH}]K_{app}}}{2K_{app}}\right)\right) \quad (1)$$

The solid lines in Figure 1 show the theoretical fits of the data to eq 1, derived for Scheme 1, using the values of K_{app} (M^{-1}) and $(k_{Cd})_{app}$ (M^{-1} s^{-1}) obtained by nonlinear least-squares analysis (Table 1), where K_{app} and $(k_{Cd})_{app}$ are apparent association constants and rate constants, respectively. Figure 1 shows that marked curvature is observed in these plots of k_{obsd} against [**L2OH**] for reactions at low pH (see inset), but nearly linear plots are observed for data obtained at high pH. The change in the shapes of these plots reflects the increasing values of K_{app} for formation of the dinuclear complex at high pH. The plots become linear when [**L2OH**] ≫ $[1/(2K_{app}) - ([\mathbf{L2OH}]/K_{app})^{1/2}]$ (eq 1). Note that an increase in K_{app} will cause these plots to appear linear at a lower [**L2OH**].

Table 1. Apparent Binding and Rate Constants from a Fit of the Data in Figure 1 to Eq 1

pH	K_{app} (M^{-1})	$(k_{Cd})_{app}$ (M^{-1} s^{-1})
8.02	1.6×10^3	0.31
8.43	4.8×10^3	0.74
8.93	1.2×10^4	2.3
9.31	3.9×10^4	2.8
10.00	1.2×10^5	3.5
10.52	1.2×10^5	3.5

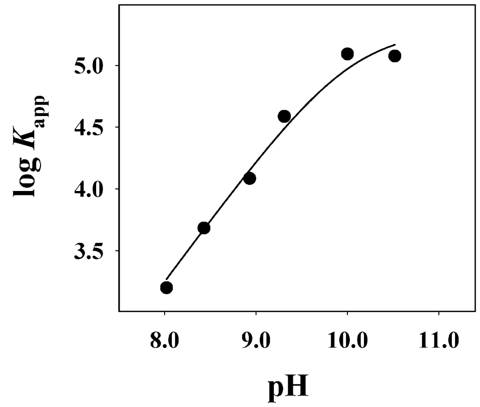
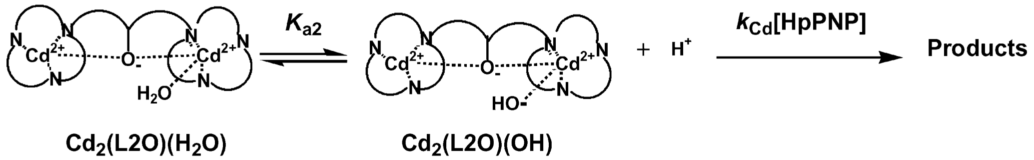


Figure 2. pH dependence of the binding of Cd(II) to Cd**(L2OH)**. The solid line represents the nonlinear least-squares fit of the data from Table 1 (K_{app}) to eq 2.

Figure 2 shows the dependence of K_{app} (Table 1) on pH. The solid line through the data provides the nonlinear least-squares fit of log K_{app} to the logarithmic form of eq 2 derived from Scheme 2 using pK_{a1} = 10.0 for deprotonation of the mononuclear complex and K = 200 000 M^{-1} for binding of Cd(II) to the deprotonated mononuclear complex. The uncertainty of the values of K_{app} obtained by fitting of the kinetic data increases as the plots from Figure 1 become nearly linear at high pH, because under these conditions there is nearly quantitative conversion of **L2OH** to the dinuclear complex even at the lowest concentration of ligand examined. Therefore, while the fit of the data from Figure 2 to eq 2 is in good agreement with spectroscopic results which show that formation of the dinuclear complex is pH dependent and is complete only at high pH, the values of pK_{a1} = 10.0 and K = 200 000 M^{-1} obtained from this fit are only approximate.

$$K_{app} = \frac{KK_{a1}}{K_{a1} + [\mathrm{H}^+]} \quad (2)$$

Scheme 3



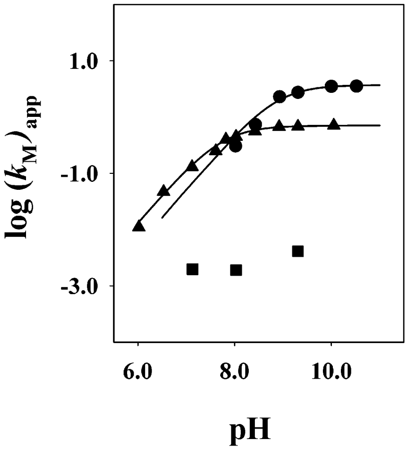


Figure 3. pH−rate profiles of log of the apparent second-order rate constant $(k_M)_{app}$ for cleavage of HpPNP catalyzed by dinuclear **L2OH** complexes of (▲) Zn(II), (●) Cd(II), and (■) Cu(II). The solid lines represent the nonlinear least-squares fit of the data to eq 3.

The mononuclear complex (Cd**(L2OH)**) is shown as a "sandwich" in Scheme 2, on the basis of the results of ^{1}H NMR studies reported here and the more extensive spectroscopic results reported for (Zn**(L2OH)**) and related complexes.[2,10] The data from Figures 1 and 2 provide evidence that loss of a proton from the mononuclear complex results in a large increase in the binding affinity of a second Cd(II). These data provide no information about the site of ionization. The ionized site is drawn as an alkoxide in Scheme 2, because we have shown that a bridging alkoxide ion provides a critical driving force that allows two Zn(II) cations to be brought together in the cationic core of (Zn_2**(L2O)**).[1,2] The complexes drawn in Scheme 2 and their role in the formation of Cd_2**(L2O)** are discussed further below.

Figure 3 shows the plot of the limiting second-order rate constants (solid circles) ($(k_{Cd})_{app}$ (M^{-1} s^{-1}); Table 1) against pH. The solid line was calculated from the logarithmic form of eq 3, derived from Scheme 3, using values of $pK_{a2} = 9.0$ and $k_{Cd} = 4.0$ M^{-1} s^{-1}. By comparison with Zn_2**(L2O)**,[1] we suggest that the ionization which activates the dinuclear Cd(II) complex for cleavage of **HpPNP** corresponds to loss of a proton from Cd_2**(L2O)**(OH_2) to form Cd_2**(L2O)**(OH). For the sake of simplicity, this diagram shows only a single ionizable metal-bound water.

(b) Cu(II) Complex. The dependence of first-order rate constants for **HpPNP** cleavage on Cu(II) complex concentration is straightforward, but the pH dependence is very different from that observed for Cd_2**(L2O)** and Zn_2**(L2O)** (Figure 3). The observed first-order rate constant for **HpPNP** cleavage at pH 8.0 is directly proportional to **[L2OH]** for experiments in which [Cu(II)] and **[L2OH]** are increased at a constant ratio of 2 to 1, and this is consistent with mass spectrometric experiments which suggest that Cu_2**(L2O)** is completely formed under these conditions. However, the second-order rate constants for **HpPNP** cleavage catalyzed by the dinuclear Cu(II) complex are independent of pH.

$$(k_{Cd})_{app} = \frac{k_{Cd}K_{a2}}{K_{a2} + [H^+]} \tag{3}$$

Structure−Activity Effects of Divalent Cations. (a) Stability of Zn_2(L2O) and Cd_2(L2O). Zn(II) and Cd(II) are isoelectronic but differ substantially in ligand−metal cation bond length and, consequently, often have different coordination numbers and geometries. We propose that the following differences in the catalytic properties and solution speciation of the Zn(II) and Cd(II) complexes reported here can be explained by the greater Lewis acidity of Zn(II), which leads to stronger binding to anionic oxygen and neutral nitrogen donors, and by the different geometric preferences of Cd(II) compared to Zn(II).

(1) The dinuclear complex of Zn(II) (Zn_2**(L2O)**) forms quantitatively at pH ≥ 6 at [Zn(II)] = 2.00 mM and [**L2OH**] = 1.00 mM.[1,2] However, quantitative conversion of 1.00 mM Cd(II) and 0.500 mM **L2OH** to Cd_2**(L2O)** is only observed at high pH > 10.0.

(2) In solutions containing a 2:1 ratio of Cd(II) to **L2OH** (1.00 mM **L2OH**) at pD 8.1, mononuclear Cd**(L2OH)** is the dominant complex.

(3) The rate law for **HpPNP** cleavage catalyzed by the catalytically active dinuclear Cd(II) complex of **L2OH** reveals a pK_a of 10.0 for ionization of a group of the inactive mononuclear Cd(II) complex that results in a large increase in the affinity of this complex for a second Cd(II) (Figure 2). We assign this pK_a to the loss of a proton from the linker hydroxyl.

These data are consistent with the conclusion that assembly of Zn_2**(L2O)** from free ligand and metal ion is enhanced compared with assembly of Cd_2**(L2O)** by the generally higher Lewis acidity of Zn(II) compared to Cd(II).[13−16] For example, a bound pendent alcohol of an azamacrocycle complex of Zn(II) ionizes with a pK_a of 1.0 log unit lower than a bound alcohol in the analogous Cd(II) complex,[17] suggesting that Zn(II) binds more tightly to the alkoxide group. In addition, the stronger binding of Zn(II) to **L1** (log $K = 11.2$) compared to binding of Cd(II) to **L1** (log $K =$

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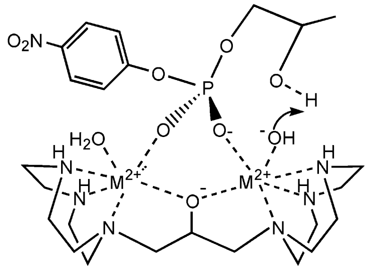
9.4)[15] is consistent with a higher overall equilibrium constant for the formation of Zn_2**(L2O)** compared to Cd_2**(L2O)**. However, it is unlikely that these differences alone would give rise to the very different speciation observed for **L2OH** complexes of Zn(II) and Cd(II). In particular, the observation of a mononuclear Cd(II) complex of **L2OH** even at 2:1 ratios of Cd(II) to **L2OH** is remarkable given that there is no evidence for a mononuclear Zn(II) complex under similar conditions.

There are at least two differences between the coordination properties of Zn(II) and Cd(II) that play an important role in controlling their speciation with **L2OH**. First, there is the higher affinity of Zn(II) for alkoxide ligands as discussed above, and this will favor the formation of the dinuclear complex. Similar to the Cd(II) system, a 1:1 ratio of Zn(II) to **L2OH** (5.00 mM **L2OH**) at neutral pH gives a stable mononuclear sandwich complex as the predominant species in solution;[2] however, unlike the Cd(II) system, a 2:1 ratio of Zn(II) to **L2OH** at neutral pH leads to the exclusive formation of the dinuclear complex. In addition, a related ligand containing two **L1** units and a propyl linker lacking an alcohol group forms a stable Zn(II) mononuclear sandwich compound that does not bind a second Zn(II) even in the presence of a 2:1 ratio of Zn(II) to ligand.[10] This suggests that the alcohol in the linker has a critical role in the formation of dinuclear **L2OH** complexes. For the Cd(II) system as well, deprotonation of the alcohol group drives the formation of Cd_2**(L2O)**.

Second, Cd(II) shows a greater propensity compared to Zn(II) to form hexacoordinate complexes. For example, it has been shown for a related binuclear ligand with eight amine donors that the preferred form of the Cd(II) complex is a mononuclear sandwich complex, whereas Zn(II) binds to form a dinuclear complex under similar conditions at neutral pH.[18] The effect of stabilizing a sandwich form of a mononuclear metal ion complex will be to increase the equilibrium constant for binding of the first metal ion and decrease the equilibrium constant for binding of the second metal ion, so that the net equilibrium constant for formation of the dinuclear complex remains constant. We report here an equilibrium constant of 200 000 M^{-1} for the binding of a second Cd(II) to the alkoxide form of the mononuclear complex (Cd**(L2O))** that is within the range of the equilibrium constants reported for binding of a second Cd(II) to ligands containing linked **L1** units.[13] However, a larger equilibrium constant is predicted for binding of a second Cd(II) to Cd**(L2O)** because binding is accompanied by formation of an alkoxide chelate. This is consistent with the notion that the equilibrium constant for binding of a second Cd(II) to **L2OH** is reduced because binding is coupled to the cleavage of a stable Cd(II) mononuclear sandwich complex.

Figure 2 provides evidence that **L2OH** coordinated to a *single* Cd(II) undergoes loss of a proton with a pK_a of 10.0. In Scheme 2, we show that the proton is lost from the alcohol group. This assignment is based on data from related complexes which show that an alcohol group has a higher pK_a than do amine groups in the formation of mononuclear Cd(II) azamacrocycles with pendent alkoxide arms.[17] For this reason, Cd**(L2O)** is drawn in Scheme 2 as a complex with a bound alkoxide and **L1** unit, although there are other possible Cd(II) alkoxide complexes that are consistent with the data. In any case, it is important to note that the value of this macroscopic pK_a will depend on the relative stabilities of the mononuclear sandwich complex (Cd**(L2OH)**) compared to the open complex (Cd**(L2O)**), if the opening of this complex is "coupled" with the loss of a proton from the linker hydroxyl (Scheme 2). Thus, the value of this ionization constant is not easily interpreted.

Scheme 4



(b) Catalytic Activity of Zn_2(L2O) and Cd_2(L2O). The dinuclear complex of Zn_2**(L2O)** is converted from an inactive to an active form upon ionization of an acidic residue with pK_a = 7.8.[1] This corresponds to the pK_a for ionization of a water ligand to form Zn_2**(L2O)(OH)**. By comparison, Cd_2**(L2O)** is converted from an inactive to an active form upon ionization of an acidic residue with pK_a = 9.0, and this is consistent with the weaker acidity expected for water bound to Cd(II) compared with Zn(II).[16,19]

There are several possible explanations for the higher catalytic activity at high pH observed for Cd_2**(L2O)**(OH) compared to Zn_2**(L2O)**(OH). The limiting second-order rate constant at high pH of 4.0 M^{-1} s^{-1} for the dinuclear Cd(II) complex is about 5-fold larger than that for the dinuclear Zn(II) complex (0.71 M^{-1} s^{-1}). This difference in activity is consistent with a mechanism in which the metal hydroxide ligand accepts a proton from the hydroxyl of the substrate to generate an alkoxide ion, which then undergoes intramolecular addition to the phosphate diester to form a cyclic phosphate (Scheme 4). Here the higher activity of the dinuclear Cd(II) complex would be due to the higher basicity of the Cd(II)-bound hydroxide ion that accepts the proton from substrate. In line with this hypothesis, a correlation between the pK_a of Zn(II) bound water and the pH-limiting first-order rate constant for cleavage of **HpPNP** has been recently reported for mononuclear Zn(II) complexes.[20] According to this relationship, the higher the pK_a of the Zn(II) bound water, the more active the catalyst at the pH-limiting value. An increase in the pK_a of Zn(II) bound water of 1.0 pH unit results in a 7.5-fold increase in the pH-limiting rate

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constant, similar to that observed here. A second possibility is that there is modest steric hindrance to the development of optimal electrostatic interactions between Zn_2**(L2O)**(OH) and substrate at the transition state for cleavage of the phosphodiester substrate and that the longer metal cation−ligand bond distances for Cd_2**(L2O)**(OH) give a more “open” complex that allows the substrate to bind more closely to the metal cation.

(c) Catalytic Activity of Cu_2(L2O). The dinuclear Cu_2**(L2O)** complex is a poor catalyst despite the fact that the bridging alkoxide linker in Cu_2**(L2O)** maintains a Cu(II)−Cu(II) distance (3.58 Å)[11] suitable for cooperative interaction of the two Cu(II) ions and sufficiently large to prevent the formation of intramolecular bridging hydroxides.[11,21,22] Comparison to efficient dinuclear Cu(II) catalysts of ligands containing **L1** units suggests that the inactivity of Cu_2**(L2O)** is due to a lack of available coordination sites for binding and catalysis. Active dinuclear Cu(II) catalysts for RNA cleavage based on linked **L1** macrocycles have two available coordination sites on each Cu(II).[23] Two coordination sites on each metal center are a sufficient number for binding the phosphate diester to both metal ion centers and for formation of a hydroxide ligand. On the basis of a recently reported crystal structure, each Cu(II) in Cu_2**(L2O)** is five-coordinate with only one available coordination site on each Cu(II) cation,[11] making it impossible to bind a phosphate diester substrate and hydroxide on the same metal ion. The analogous Zn_2**(L2O)** complex has at least three and possibly four coordination sites distributed between the two Zn(II) centers on the basis of structural data.[1] This coordination sphere allows the Zn_2**(L2O)**(OH) complex to interact with the phosphate diester and the hydroxyl group of RNA analogues **HpPNP** (Scheme 4) and **UpPNP**.[3]

Summary. Studies of dinuclear metal ion catalysts containing divalent metal ions with different geometric preferences and Lewis acidity give new insight into the properties that are important in the design of dinuclear catalysts for the cleavage of phosphate diesters. Second-order rate constants for cleavage of **HpPNP** by these dinuclear complexes at pH 9.3 vary by 3 orders of magnitude and decrease in the order Cd_2**(L2O)** (2.8 M^{-1} s^{-1}) > Zn_2**(L2O)** (0.68 M^{-1} s^{-1}) > Cu_2**(L2O)** (0.0041 M^{-1} s^{-1}). That the dinuclear Cu(II) catalyst is the least active is attributed to a lack of available coordination sites. The dinuclear Cd(II) complex is more active than the Zn(II) analogue at high pH but much less active a low pH values, in part because the complex does not form appreciably under these conditions at neutral pH. However, once the complex forms, it is a highly active catalyst, suggesting that dinuclear Cd(II) complexes may be developed as potent catalysts for the cleavage of phosphate diesters and RNA if a more suitable ligand were prepared. This may best be accomplished by the incorporation of a linker that would prevent the formation of a sandwich complex and by the use of a more easily deprotonated alcohol group or a more strongly binding bridging group such as a thiolate.

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Supporting Information Available: Additional figures with NMR spectra and a table of rate constants. This material is available free of charge via the Internet at http://pubs.acs.org.

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