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Hydrolysis of Phosphate Diesters with Copper(II) Catalysts

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Hydrolysis of phosphate diesters $(4\text{-}NO_2C_6H_4O)_2PO_2Na$ (**1**) and $(4\text{-}NO_2C_6H_4O)(CH_3CH_2O)PO_2Li$ (**2**) is catalyzed by $Cu(bpy)^{2+}$ (bpy = 2,2'-bipyridine) in aqueous solution at 75 °C in the pH range 5.8-8.3. Greater than 1000 turnovers and 200 turnovers per $Cu(bpy)^{2+}$ are observed in the hydrolysis of **1** and **2**, respectively. Catalytic rate enhancements of the hydrolysis of **1** and **2** by 1×10^{-3} M $Cu(bpy)^{2+}$ at pH 6.5 over spontaneous hydrolysis under the same conditions without catalyst are 2000 and 150, respectively. The hydrolysis of copper-bound **2** proceeds 6300-fold more rapidly (pH 7.85) than hydrolysis of **2** in the absence of catalyst. Kinetics for the $Cu(bpy)^{2+}$-catalyzed hydrolysis of **2** are examined in detail. The pH-rate profile indicates two reaction pathways, hydrolysis with $Cu(bpy)(OH)^+$ or its kinetic equivalent as the active catalyst at alkaline pH and less effective hydrolysis by $Cu(bpy)^{2+}$ at low pH. Saturation kinetics follow Michaelis-Menton behavior ($K_m = 4.7 \times 10^{-2}$ M, $k_{cat} = 5.6 \times 10^{-4}$ s^{-1}) and provide evidence for the formation of a copper-phosphate ester complex, which decays to products. Labeling studies in $^{18}OH_2$ show no incorporation of ^{18}O into *p*-nitrophenol. A single ^{18}O label incorporates into the $(C_2H_5O)PO_3^{2-}$ product. Several simple transition-metal complexes promote the catalytic hydrolysis of phosphate diesters **1** and **2**, although none are as effective as $Cu(bpy)^{2+}$. Second-order rate constants for $Cu(bpy)^{2+}$-promoted hydrolysis in the series of 4-nitrophenyl phosphate esters (triester, diester (anion), monoester (dianion)) vary by only a factor of 60 in contrast to those for the reaction of these phosphate esters with anionic nucleophiles in the absence of metal catalysts, which show large differences in second-order rate constants ($>10^3$) between each ester in the series.

Introduction

Restriction endonucleases have played a crucial role in the development of molecular genetics and genetic engineering. Recent studies have focused on understanding the mechanism of base-sequence selective recognition, which is characteristic of these endonucleases.[1] The mechanisms employed by endonucleases to cleave the phosphodiester bonds of DNA are also under investigation; these often include the feature of phosphate ester activation by divalent metal cations. One well-studied example is the role of the calcium ion in the active site of staphylococcal nuclease.[2,3] A contribution of $10^{4.6}$ to the overall rate enhancement of enzymatic hydrolysis has been estimated for this metal cation.[4] The need for an assessment of such mechanisms in well-defined model systems led us to examine the hydrolysis of simple phosphate diesters by metal complexes.

Remarkably few reports exist in the literature concerning the hydrolysis of phosphate diesters by homogeneous transition-metal complexes, and none of these metal complexes exhibit catalytic turnover. This contrasts with the numerous metal-based model systems reported for the hydrolysis of phosphate monoesters,[5,6] triesters,[7,8] fluorophosphates,[9] fluorophosphonates,[9] and phosphoric anhydrides.[10,11] Examples of phosphate diester hydrolysis by metal complexes in homogeneous solution generally belong to a special class of functionalized esters containing a neighboring group that participates in hydrolysis or a cyclic phosphate diester.[12a] These include metal-cation-accelerated hydrolysis of salicylic acid *o*-aryl phosphates[12b] and the hydrolysis of the phosphate diester bonds in RNA, which are labilized by the 2-hydroxy substituent of ribose.[13] Both nonmetallic micelles[14] and metallomicelles[15] accelerate the hydrolysis of simple phosphate diesters.

The paucity of model catalytic systems perhaps results from the robust nature of phosphate diesters.[16] The monoanionic form of the diester, which predominates at pH >2, resists attack by anionic nucleophiles.[17a] It does not react in a dissociative manner as has been proposed for the monoanion and dianion of phosphate monoesters.[18] The exceptional stability of phosphate diesters has been suggested as one reason that nucleic acids evolved as genetic material.[19] A potential application for a catalyst that hydrolyzes phosphodiester bonds would be as a tool for molecular genetics. Synthetic sequence-specific binding agents have been attached to a Fenton reagent system to cut DNA with sequence selectivity.[20] Unfortunately, the cutting event relies on free hydroxyl radical, which attacks the DNA in an as yet undefined reaction. Development of a catalyst that hydrolyzes phosphate diester bonds would be the preferred method for cutting DNA.

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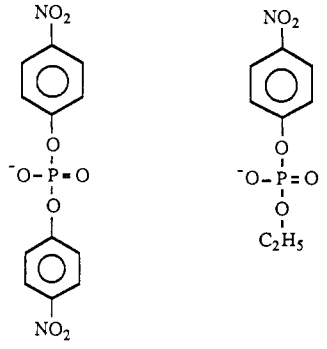
The search for antitumor drugs[21] and small-molecule probes of DNA structure[22] has focused attention on the interaction between metal complexes and nucleic acids. Although the majority of these studies involve metal coordination to the nitrogenous bases of DNA or intercalation of ligands between stacked bases, other studies show that the phosphodiester backbone of DNA may be a target of metal complexes. For example, Cp_2VCl_2, a member of a class of early-transition-metal metallocene dihalides that show antitumor activity, interacts selectively in solution with the phosphate ester of nucleotides.[23] Zinc complexes of nucleotides, methylated at the phosphate ester, exhibit either metal–base coordination[24] or phosphate diester coordination.[25] Finally, it has been reported that $Ba(OH)_2$ in strongly alkaline media[26a] and amine complexes of Cu(II), Zn(II), Co(II), Cd(II), and Pb(II) under mild conditions[26b] hydrolyze the phosphate diester bonds of DNA. Several metal salts that are carcinogens produce DNA strand breakage, but the site of cleavage is not known.[27] In these studies the site of initial cleavage is difficult to determine because of problems inherent in studying the products of strand breakage in large DNA molecules. Again, there is a clear need for model studies to examine the reactivity of phosphate diesters with metal complexes.

We present kinetic studies of the catalytic hydrolysis of two phosphate diesters (**1** and **2**) by $Cu(bpy)^{2+}$, where 2,2′-bipyridine = bpy, in aqueous solution and show that several metal salts



1 **2**

accelerate hydrolysis of these phosphate esters. Mechanistic studies on the $Cu(bpy)^{2+}$-catalyzed hydrolysis of a mixed aryl–alkyl phosphate are discussed. The rates of hydrolysis promoted by $Cu(bpy)^{2+}$ for the series phosphate triester, diester, and monoester are compared.

Experimental Section

Disodium 4-nitrophenyl phosphate (Sigma), bis(4-nitrophenyl) phosphate (free acid, Sigma), reagent grade inorganic salts, and Sigma buffers MES (2-morpholinoethanesulfonic acid), HEPES (*N*-(2-hydroxyethyl)piperazine-*N*′-ethanesulfonic acid), EPPS (*N*-(2-hydroxyethyl)piperazine-*N*′-propanesulfonic acid), CHES (2-(cyclohexylamino)ethanesulfonic acid), and tren (tris(aminoethyl)amine) were purchased from commercial sources and used without purification. The 2,2′-bipyridine ligand was recrystallized twice from hexanes and dried in vacuo. The phosphate triester 4-nitrophenyl diethyl phosphate was prepared according to literature methods[17a] and distilled in vacuo (~1 Torr at 140–142 °C). The diester lithium 4-nitrophenyl ethyl phosphate[17b] was prepared by treating an acetone solution of the triester with lithium chloride and refluxing the solution overnight. Addition of a 1:2 mixture of hexanes and diethyl ether to the cooled acetone solution yielded the solid diester on standing overnight. The free acid of bis(4-nitrophenyl) phosphate was recrystallized from an ethanol–water mixture, and the lithium salt of 4-nitrophenyl ethyl phosphate was recrystallized from ethanol–acetone. Ethyl phosphate was prepared according to literature methods,[28] isolated as the calcium salt, and converted to the disodium salt. All phosphate esters were analyzed by spectrophotometric measurement of the *p*-nitrophenolate released on complete acid hydrolysis. All solutions were prepared with Fisher HPLC grade water.

The concentration of $Cu(NO_3)_2$ was determined by titration against ethylenediaminetetraacetic acid with murexide[29] as an indicator. The acid dissociation constant of *p*-nitrophenol at 75 °C was determined to be 2.14×10^{-7} by pH measurements made during titration of the *p*-nitrophenol against a NaOH standard prepared by dilution of J. T. Baker carbonate-free concentrate with CO_2-free water. Titrations were performed in a water-jacketed cell under nitrogen.

An Orion research digital ion analyzer 501, equipped with a temperature compensation probe, was used for pH measurements. All ^{31}P NMR spectra were recorded with use of a General Electric QE 300-MHz spectrometer. Chemical shifts are reported relative to external 85% phosphoric acid. A Varian 3400 gas chromatograph with flame ionization detector was employed for detection of ethanol. Gas chromatograph–mass spectrometry analyses were performed at the University of California at Riverside facility by using a Supelcoport SP 2100 10% capillary column. Kinetic measurements were made with use of an IBM 9420 UV–vis spectrometer equipped with a thermostated cell compartment.

^{18}O Labeling Study. A 5-mL 20% $^{18}OH_2$ solution 0.2 M in lithium 4-nitrophenyl ethyl phosphate and 5 mM in $Cu(bpy)^{2+}$ was heated at 75 °C for 17 days, and the pH of the solution was maintained between 6.5 and 7.5 by periodic addition of NaOH. The solution was diluted to 50 mL, the pH was adjusted to 9, and the solution was passed through a Sephadex SP C-25 column (Na^+ form, 1.5 × 10 cm) to absorb cationic species. The eluant was reduced in volume to 2 mL and filtered, and a few drops were analyzed by ^{31}P NMR spectroscopy (D_2O, pH 9). The pH of the eluant was adjusted to ~0 with concentrated HCl, and the water was removed in vacuo. The ethanol-soluble fraction of the resulting white solid was analyzed by gas chromatography–mass spectrometry. Experiments were duplicated. A third experiment consisting of hydrolysis of a 25% $^{18}OH_2$ solution 0.02 M in lithium 4-nitrophenyl ethyl phosphate and 0.01 M in $Cu(bpy)^{2+}$ at 75 °C for 4 days was worked up as above and analyzed solely by ^{31}P NMR spectroscopy. High-resolution ^{31}P NMR spectra, for detection of peaks shifted from ^{18}O incorporation, were recorded with a sweep width of 1000 Hz and were modified by a double exponential multiplier of 6 containing Gaussian multiplier and negative line-broadening components (QE 300 Charm software).

Product Analysis. A 5-mL solution 0.1 M in lithium 4-nitrophenyl ethyl phosphate and 0.05 M in $Cu(bpy)^{2+}$ was heated at 75 °C in a sealed container until hydrolysis was complete. This solution was analyzed for ethanol by gas chromatography, followed by treatment with Sephadex SP C-25 ion-exchange resin as described above to remove catalyst, and analyzed by ^{31}P NMR spectroscopy and gas chromatography–mass spectrometry.

Kinetics. The initial rate of production of *p*-nitrophenolate was monitored spectrophotometrically at 400 nm. Reactions performed at pH <9 were corrected for the degree of ionization of *p*-nitrophenolate at 75 °C. The Sigma biological blood buffers MES, HEPES, EPPS, and CHES were used to maintain a constant pH in the range 6.0–9.0. The pH of solutions was adjusted with NaOH or HNO_3 and checked at 75 °C.

First-order rate constants were calculated from the slopes of the linear plots of optical density against time by converting to concentration units (ϵ = 18 700) and dividing by the initial phosphate ester concentration. Rate constants for the hydrolysis of bis(4-nitrophenyl) phosphate were determined as described[30] to allow for hydrolysis of 4-nitrophenyl phosphate. All experiments were run in duplicate or triplicate, and tabulated data represent the average of these experiments. Standard deviations were in the range 2–5%. Error in rate constants was conservatively

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Table I. Representative Pseudo-First-Order Rate Constants for the Hydrolysis of Sodium Bis(4-nitrophenyl) Phosphate (**1**) and Lithium Ethyl 4-Nitrophenyl Phosphate (**2**) in Water at 75 °C[a]

substrate		10^6k_0, s^{-1}	pH
1	$Cu(bpy)^{2+}$ (1 mM)	42	6.50
1	NaOH (10 mM)	9.8	10.80
1	control	0.021	6.50
2	$Cu(bpy)^{2+}$ (1 mM)	7.4	6.50
2	NaOH (10 mM)	17	10.80
1	$Ni(tren)^{2+}$ (1 mM)	3.6	8.60
1	$Cu(NO_3)_2$ (0.1 mM)	0.57	6.50
1	$Ni(NO_3)_2$ (1 mM)	0.13	6.60
1	$Mg(NO_3)_2$ (1 mM)	0.18	8.20
2	control	0.089	7.50
2	control	0.049	6.50

[a] pH measurements made at 75 °C with temperature compensation probe; μ = 0.1 M ($NaNO_3$), 0.01 M buffer; bpy = 2,2′-bipyridine, tren = tris(aminoethyl)amine; $K_w = 2.0 \times 10^{-13}$. Rate constants were obtained by averaging three or more kinetic experiments. Standard deviations were less than 5% of the rate constants listed.

Table II. Kinetic Data for the Hydrolysis of Lithium Ethyl 4-Nitrophenyl Phosphate by $Cu(bpy)^{2+}$ in Water at 75 °C with Variation of the 2,2′-Bipyridine to Copper(II) Ratio[a]

[bpy], M	10^7k_0, s^{-1}	[bpy], M	10^7k_0, s^{-1}
0	0.74	1.0×10^{-4}	3.5
2.5×10^{-5}	2.9	2.0×10^{-4}	2.3
5.0×10^{-5}	5.5	5.0×10^{-4}	1.5

[a] bpy = 2,2′-bipyridine; pH 7.85, 0.01 M HEPES, μ = 0.1 M ($NaNO_3$), 5×10^{-5} M $Cu(NO_3)_2$.

estimated from uncertainties introduced by temperature, pH, and stock concentration fluctuations, since the least-squares fitting errors were negligible. Generally, second-order rate constants were obtained from plots of first-order rate constants against catalyst concentration. Rates of hydrolysis of phosphate esters in the absence of catalyst were measured and, when not negligible, were subtracted from the rate of catalytic hydrolysis.

Results

Representative pseudo-first order rate constants are tabulated for the hydrolysis of **1** and **2** by $Cu(bpy)^{2+}$, several metal salts, and sodium hydroxide in aqueous solution at 75 °C (Table I). (Aquo ligands are omitted from the formulas of metal complexes for simplicity.) The spontaneous hydrolyses of **1** and **2** are not buffer catalyzed under these conditions and are tabulated for comparison. One would like to compare second-order rate constants for the hydrolytic agents in Table I, as an index of their efficiency in phosphate ester hydrolysis. However, the alkaline hydrolyses of **1** and **2** are not first order in sodium hydroxide[31a] nor are they first order in $Cu(bpy)^{2+}$ at millimolar concentrations. We will discuss this further in the Discussion in the context of reported rate enhancements.

The observation of pseudo-zeroth-order kinetics for several turnovers of $Cu(bpy)^{2+}$ in buffered solutions containing an excess of phosphate ester **1** or **2** confirmed that the copper complex was functioning catalytically. No rate decrease in the hydrolysis of **1** by $Cu(bpy)^{2+}$ was observed after greater than 1000 turnovers. Although 200 turnovers were measured in the hydrolysis of **2** by $Cu(bpy)^{2+}$, the rate of hydrolysis began to decrease after ~50 turnovers. Addition of ethyl phosphate in excess over **2** did not inhibit hydrolysis of **2** by $Cu(bpy)^{2+}$.

Ethyl phosphate and *p*-nitrophenolate are produced in the hydrolysis of **2** by $Cu(bpy)^{2+}$. The absence of ethanol in hydrolyzed solutions of **2** (by gas chromatography measurements) showed that the product ethyl phosphate is not hydrolyzed, nor is ethanol produced from the phosphate diester, in the presence of $Cu(bpy)^{2+}$. This was corroborated by the absence of inorganic phosphate or 4-nitrophenyl phosphate determined by using ^{31}P NMR spectroscopy. Inorganic phosphate was detected by using ^{31}P NMR spectroscopy for the long-term hydrolysis of **1** at neutral pH by $Cu(bpy)^{2+}$, and the rate of hydrolysis of 4-nitrophenyl phosphate (**3**) was accelerated by $Cu(bpy)^{2+}$ (Table V). Since reaction kinetics were followed by measuring the production of *p*-nitrophenolate, we chose to avoid the complications of a potential secondary hydrolysis by using the mixed aryl–alkyl ester **2** for detailed studies of phosphate diester hydrolysis. Rate constants for the hydrolysis of **1** by $Cu(bpy)^{2+}$, and by other metal complexes, were corrected for phosphate monoester hydrolysis as detailed in the Experimental Section.

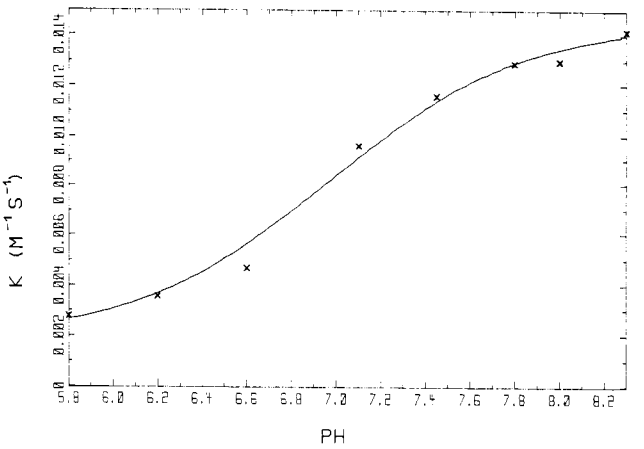

Figure 1. Dependence of second-order rate constant, k, on pH for the hydrolysis of **2** by $Cu(bpy)^{2+}$ (75 °C, μ = 0.1 M ($NaNO_3$), 5 mM buffer). The data are fitted to a theoretical curve with $pK_a = 7.0$, $k_1 = 1.9 \times 10^{-3}$ M^{-1} s^{-1}, and $k_2 = 1.4 \times 10^{-2}$ M^{-1} s^{-1}.

Nitrate salts of Cu^{2+}, Ni^{2+}, and Mg^{2+} also modestly accelerated the rate of phosphate diester hydrolysis (Table I). Phosphate diester hydrolyses promoted by Ni^{2+} could not be run at alkaline pH because of the formation of precipitates. Both Mg^{2+} and $Ni(tren)^{2+}$ (tren = tris(aminoethyl)amine) showed no activity at neutral pH but promoted hydrolysis at alkaline pH. Of the simple inorganic salts only Cu^{2+} exhibited catalytic behavior; however, $Ni(tren)^{2+}$ showed catalytic turnover in the hydrolysis of **1**. Inspection of Table II shows that one bipyridine ligand coordinated to Cu^{2+} promoted the hydrolysis of **2** 7-fold over hydrolysis promoted by Cu^{2+}. Coordination of a bipyridine ligand also prevented precipitation of copper hydroxide. Alkaline solutions of **2** at Cu^{2+} concentrations greater than 2×10^{-4} M in the absence of bipyridine invariably formed precipitates. Similarly, addition of tren to Ni^{2+} solutions containing **1** prevented formation of precipitates above neutral pH.

The following copper complexes are present in an aqueous solution of Cu^{2+} and 2,2′-bipyridine in a 1:1 ratio and may contribute to copper-catalyzed hydrolysis of **2**:

$$k_0 = k_1[Cu(bpy)^{2+}] + k_2[Cu(bpy)(OH)^+] + k_3[Cu(bpy)_2^{2+}] + k_4[(Cu(bpy)(OH))_2^{2+}] + k_5[Cu^{2+}] \quad (1)$$

The concentrations[32] of these copper complexes at various 2,2′-bipyridine to copper ratios are listed in Table III. At a 1:1 ratio of Cu^{2+} to 2,2′-bipyridine (5×10^{-5} M, pH 7.85) hydrolysis of **2** by Cu^{2+} ($k_5 = 1.5 \times 10^{-3}$ M^{-1} s^{-1}; Table II) or by $Cu(bpy)^{2+}$ ($k_1 = 1.9 \times 10^{-3}$ M^{-1} s^{-1}; see below) is insignificant (<1% of the observed rate of hydrolysis). That the rate of hydrolysis of **2** at 2,2′-bipyridine to Cu^{2+} ratios greater than 1.0 tracks the concentration of $Cu(bpy)(OH)^+$ in solution suggests that $Cu(bpy)_2^{2+}$ does not promote hydrolysis of **2**.

The pH profile (Figure 1) of $Cu(bpy)^{2+}$-catalyzed hydrolysis of **2** show two pH-independent plateaus and an intermediate pH range, where the rate of hydrolysis depends on pH. The data can

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Table III. Effect of the Concentration (M) of 2,2′-Bipyridine on the Concentrations (M) of Copper(II) Complexes in Water at 75 °C[a]

[bpy]	$[Cu^{2+}]$	$[Cu(bpy)^{2+}]$	$[Cu(bpy)_2^{2+}]$	$[Cu(bpy)(OH)^{+}]$	$[(Cu(bpy)(OH))_2^{2+}]$
2.5×10^{-5}	2.5×10^{-5}	1.5×10^{-6}	1.1×10^{-9}	9.1×10^{-6}	7.2×10^{-6}
5.0×10^{-5}	6.7×10^{-7}	2.2×10^{-6}	9.3×10^{-8}	1.4×10^{-5}	1.6×10^{-5}
1.0×10^{-4}	7.5×10^{-7}	2.1×10^{-6}	7.1×10^{-6}	1.3×10^{-5}	1.4×10^{-5}
2.0×10^{-4}	2.0×10^{-9}	1.7×10^{-6}	1.8×10^{-5}	1.1×10^{-5}	9.8×10^{-6}

[a] bpy = 2,2′-bipyridine; pH 7.85; $[Cu^{2+}] = 5.0 \times 10^{-5}$ M, calculated from equilibrium constants in ref 33 and 42 as described in the supplementary material.[32,35]

Table IV. Kinetic Data for the Hydrolysis of Lithium Ethyl 4-Nitrophenyl Phosphate by $Cu(bpy)^{2+}$ in Water at 75 °C with Dependence on Catalyst Concentration[a]

$[Cu(bpy)^{2+}]$, M	$10^6 k_0$, s^{-1}	turnover rate, turnover h^{-1}
1.0×10^{-5}	0.11	0.19
2.5×10^{-5}	0.27	0.19
5.0×10^{-5}	0.54	0.19
1.0×10^{-4}	0.90	0.17
2.5×10^{-4}	2.0	0.14
5.0×10^{-4}	3.7	0.13
1.0×10^{-3}	5.1	0.092
2.5×10^{-3}	8.6	0.062

[a] bpy = 2,2′-bipyridine; pH 7.85, 0.01 M HEPES, μ = 0.1 M ($NaNO_3$).

Table V. Kinetic Data for the Hydrolysis of Disodium 4-Nitrophenyl Phosphate (**3**) in Water at 75 °C[a]

pH	$[Cu(bpy)^{2+}]$, mM	[3], mM	$10^5 k_0$, s^{-1}
6.50	5.00	1.00	4.8
6.50	2.50	1.00	2.3
6.50	1.00	1.00	0.97
6.50	5.00	2.50	4.9
6.50	5.00	5.00	5.0
6.50	0.00	1.00	0.93
8.00	5.00	1.00	1.5
8.00	2.50	1.00	0.92
8.00	1.00	1.00	0.55
8.00	5.00	2.50	1.6
8.00	5.00	5.00	1.5
8.00	0.00	1.00	0.30

[a] μ = 0.1 M ($NaNO_3$), 0.01 M HEPES or MES; bpy = 2,2′-bipyridine.

be fit to an expression for the hydrolysis of **2** by $Cu(bpy)^{2+}$ and $Cu(bpy)(OH)^{+}$ as in eq 2 and 3, where $[Cu(bpy)^{2+}]_T$ is the total

$$k_0 = k_1[Cu(bpy)^{2+}] + k_2[Cu(bpy)(OH)^{+}] \quad (2)$$

$$k_0 = \left(\frac{k_1[H^+] + k_2K_a}{[H^+] + K_a}\right)[Cu(bpy)^{2+}]_T \quad (3)$$

concentration of copper complex, K_a is the acid dissociation constant for the coordinated water molecule, k_2 is the second-order rate constant for $Cu(bpy)(OH)^{+}$-mediated hydrolysis of **2**, and k_1 is the second-order rate constant for $Cu(bpy)^{2+}$-mediated hydrolysis of **2**. A least-squares fitting of the data[34] gave pK_a = 7.0 (±0.05), k_1 = 1.9 (±0.2) × 10^{-3} M^{-1} s^{-1}, and k_2 = 1.4 (±0.1) × 10^{-2} M^{-1} s^{-1}. All other hydrolysis measurements were performed on the alkaline pH plateau where hydrolysis of **2** by $Cu(bpy)^{2+}$ is insignificant.

The rate of hydrolysis of **2** shows a first-order dependence on $Cu(bpy)^{2+}$ concentration at low concentrations of complex (Table IV and Figure 2 inset). At concentrations greater than 5×10^{-5} M the reaction order in $Cu(bpy)^{2+}$ decreases below 1 (Figure 2). This is consistent with the known[33,35] dimerization to the hydroxy-bridged dimer $[Cu(bpy)(OH)]_2^{2+}$, which is inactive as a catalyst. Calculations[32] based on estimated equilibrium constants suggest that the dimer predominates in alkaline solution even at low Cu^{2+} concentrations (5×10^{-5} M; Table III); however, the

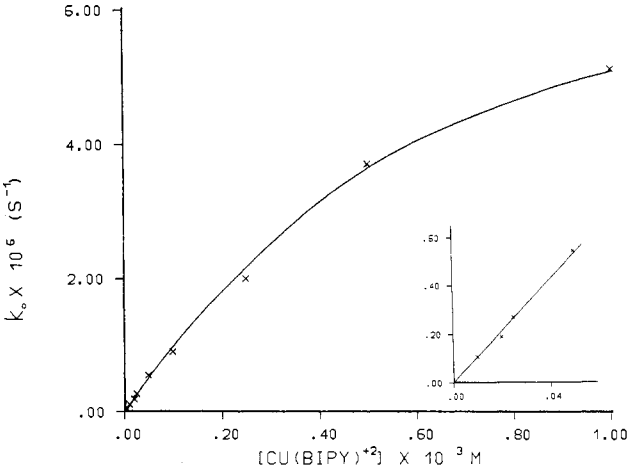


Figure 2. Dependence of k_0 for the hydrolysis of **2** on $Cu(bpy)^{2+}$ concentration. (See Table II for experimental conditions.) Inset is of data points at low $Cu(bpy)^{2+}$ concentrations fit by linear least squares to the line shown (correlation coefficient 0.994).

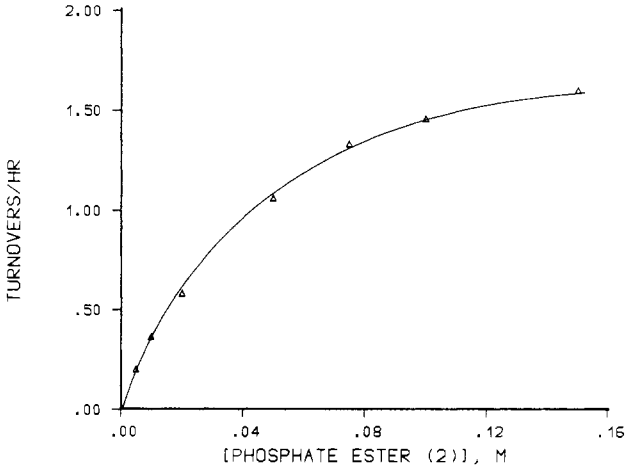


Figure 3. Dependence of the rate of hydrolysis of **2** in turnovers/h, catalyzed by $Cu(bpy)^{2+}$, on the concentration of **2** (75 °C, μ = 0.1 M ($NaNO_3$), 0.02 M EPPS, $[Cu(bpy)^{2+}] = 4 \times 10^{-5}$ M, pH 7.85).

first-order dependence on catalyst concentration observed here shows that dimerization is not significant at these concentrations.

A slight inhibition in the rate of hydrolysis (30%) of **2** by $Cu(bpy)^{2+}$ was observed on increasing the concentration of HEPES or CHES buffer from 0.001 to 0.01 M. Minor differences in the rates of hydrolysis were observed for solutions at the same pH with different buffers. Buffers that were primary amines strongly inhibited hydrolysis of **1** or **2** by $Cu(bpy)^{2+}$. This inhibition, probably by coordination of buffer to the catalyst, prohibited us from determining if general-base catalysis occurs in these hydrolyses.

The hydrolysis of **2** by $Cu(bpy)^{2+}$, initially first order in **2**, becomes independent of the concentration of **2** at high ratios of **2** to $Cu(bpy)^{2+}$. A maximum of 1.6 turnovers h^{-1} is observed. A Michaelis constant (K_m) of 4.7 (±0.2) × 10^{-2} M and a first-order rate constant (k_{cat}) for the decomposition of the intermediate copper(II) hydroxide phosphate ester complex **5** (Scheme I) of 5.6 (±0.2) × 10^{-4} s^{-1} were determined from a least-squares analysis of an Eadie–Hofstee plot (Figure 4). Hence, at low concentrations

(34) Bevington, P. *Data Reduction and Error Analysis for the Physical Sciences*; McGraw-Hill: New York, 1969.
(35) Guastafson, R. L.; Martell, A. E. *J. Am. Chem. Soc.* **1959**, *81*, 525–529.

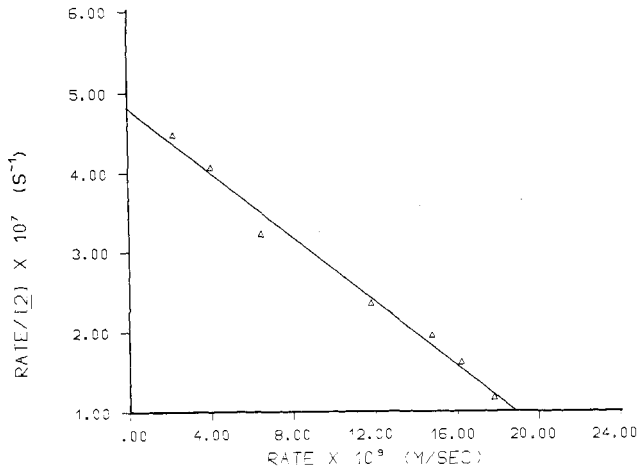


Figure 4. Eadie–Hofstee plot of data in Figure 3. rate/[2] = k_{cat}[Cu(bpy)$^{2+}$]/K_m − rate/K_m (k_{cat} = 5.6 × 10^{-4} s^{-1}, K_m = 4.7 × 10^{-2} M, correlation coefficient 0.940).

Scheme I

$$Cu(bpy)(H_2O)_2^{2+} + OH^- \rightleftharpoons Cu(bpy)(OH)(H_2O)^+ + H_2O$$

$$Cu(bpy)(OH)(H_2O)^+ + \mathbf{2} \underset{k_d}{\overset{k_f}{\rightleftharpoons}} Cu(bpy)(OH)(\mathbf{2}) + H_2O$$

$$Cu(bpy)(OH)(\mathbf{2}) \xrightarrow[k_{cat}]{} Cu(bpy)(H_2O)_2^{2+} + CH_3CH_2OPO_3^{2-} + 2H^+ + p\text{-}NO_2C_6H_4O^-$$

$$\frac{d[p\text{-}NO_2C_6H_4O^-]}{dt} = \frac{k_{cat}[Cu(bpy)(H_2O)_2^{2+}][\mathbf{2}]}{K_m + [\mathbf{2}]}$$

$$K_m = \frac{k_d + k_{cat}}{k_f}$$

of **2** the second-order rate constant for hydrolysis of **2** by Cu(bpy)(OH)$^+$ can be expressed as $k_2 = k_{cat}/K_m$. Saturation was not reached in the hydrolysis of **1** by Cu(bpy)$^{2+}$; however, comparable concentrations of phosphate ester could not be used because of the reduced solubility of **1**. A simple first-order dependence on **1** was observed.[30]

Isotopic labeling experiments were performed to determine whether hydrolysis occurred by C–O or P–O bond cleavage. Hydrolysis of **2** by Cu(bpy)$^{2+}$ in 20% $^{18}OH_2$ was stopped at ca. 50% completion, and the products were analyzed by ^{31}P NMR spectroscopy and by gas chromatography–mass spectrometry. The ^{31}P NMR peak assigned to ethyl phosphate showed a satellite 0.025 ppm upfield that was 17 ± 2% of the total monoester product. The satellite peak increased in area to 24 ± 2% of the total monoester product when the hydrolysis of **2** by Cu(bpy)$^{2+}$ was repeated in 25% $^{18}OH_2$ (Figure 5). The high-field peak was assigned to ethyl phosphate with an ^{18}O label on the phosphorus; the magnitude and direction of this shift resemble those found in other ^{18}O-labeled monophosphate esters.[7b,36] Double labeling of product was not seen in the ^{31}P NMR spectra. The ^{31}P resonance assigned to unreacted **2** showed no peak for ^{18}O incorporation. Analysis of *p*-nitrophenol by mass spectrometry showed no ^{18}O enrichment within experimental error. From these data, it can be concluded that hydrolysis proceeds predominantly (>95%) by P–O bond cleavage. This contrasts with the results for simple base hydrolysis of **2**, where substantial C–O bond cleavage is observed.[37a]

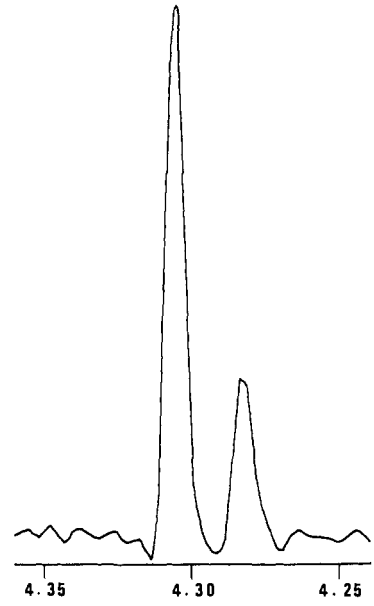


Figure 5. ^{31}P NMR spectrum of (EtO)PO_3^{2-} produced by Cu(bpy)$^{2+}$-catalyzed hydrolysis of **2** in 25% $^{18}OH_2$, showing unlabeled phosphate ester (major peak) and mono-^{18}O-labeled phosphate ester (minor peak 0.025 ppm upfield) in a ca. 3:1 ratio.

Table VI. Apparent Second-Order Rate Constants for the Hydrolysis of Phosphate Esters by Cu(bpy)$^{2+}$ [a] at 75 °C

phosphate ester	pH	10^2k, M^{-1} s^{-1}
(4-$NO_2C_6H_4O)_2PO_2Na$ (**1**)	8.0	2.0 (±0.1)
(4-$NO_2C_6H_4O$)(CH_3CH_2O)PO_2Li (**2**)	8.0	1.1 (±0.1)
(4-$NO_2C_6H_4O)PO_3Na_2$ [b] (**3**)	6.1	1.7 (±0.2)
(4-$NO_2C_6H_4O)PO_3Na_2$ [c] (**3**)	8.0	0.64 (±0.04)
(4-$NO_2C_6H_4O$)($CH_3CH_2O)_2PO$ (**4**)	8.0	36 (±1)

[a] 0.01 M HEPES or MES, μ = 0.1 M ($NaNO_3$); based on first-order rate constants for Cu(bpy)$^{2+}$ concentrations 1 × 10^{-5} to 1 × 10^{-4} M. [b] Based on first-order rate constants for Cu(bpy)$^{2+}$ concentrations 5 × 10^{-4} to 5 × 10^{-3} M. [c] Based on Cu(bpy)$^{2+}$ concentration 5 × 10^{-4} M.

The rate of hydrolysis of the monoester disodium 4-nitrophenyl phosphate (**3**) was accelerated by Cu(bpy)$^{2+}$ and showed a first-order dependence on **3** at pH 8.0 and 6.5 (Table V). As in the hydrolysis of **2** by Cu(bpy)$^{2+}$, the rate dependence of the hydrolysis of **3** on catalyst concentration under alkaline conditions is less than first order at millimolar concentrations of Cu(bpy)$^{2+}$ because the catalyst dimerizes.[33,35] Modest rate enhancements were observed because of the much higher rate of spontaneous hydrolysis of **3**, as compared to those of the diesters **1** and **2** (Table I and V). This precluded determining whether the hydrolysis was catalytic in Cu(bpy)$^{2+}$. A pH–rate profile could not be experimentally determined for **3** at concentrations where monomeric copper complexes predominate. The apparent second-order rate constant at pH 8.0 measured at 5 × 10^{-4} M Cu(bpy)$^{2+}$ is approximate since the Cu(bpy)$^{2+}$ dependence was less than 1 (Tables V and VI). However, this value is only ca. 30% low based on the dependence of the hydrolysis of **2** on Cu(bpy)$^{2+}$ concentration (Table IV).

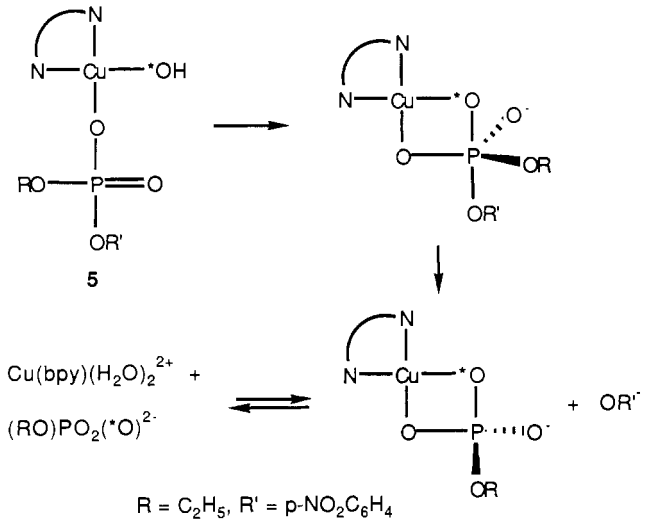
Kinetic studies[37b] for the hydrolysis of 4-nitrophenyl diethyl phosphate (**4**) by Cu(bpy)$^{2+}$ feature a first-order dependence on both **4** and catalyst at less than 1 × 10^{-4} M Cu(bpy)$^{2+}$. A sigmoidal pH–rate dependence curve resembling that for **2** was observed. The second-order rate constant on the alkaline pH plateau is listed in Table VI for comparison.

Discussion

Catalytic Efficiency. Before discussing the rate data, we first mention methods used in the calculation of catalytic rate enhancements in phosphate ester hydrolysis. Because of the slow rates of hydrolysis of phosphate monoesters, diesters, and triesters

(36) Gorenstein, D.; Taira, K. *J. Am. Chem. Soc.* **1982**, *104*, 6130–6132.
(37) (a) At 0.1 M NaOH, 75 °C and I = 0.1 M, 70 ± 3% P–O bond cleavage occurred as determined by integration of ^{31}P NMR resonances of ^{18}O-labeled product phosphate monoester and of unlabeled phosphate monoester. NaOH hydrolysis of bis(nitrophenyl) methyl phosphate occurs by C–O and by P–O bond cleavage in approximately equal proportions.[17a] (b) Morrow, J. R., Trogler, W. C., manuscript in preparation.

Scheme II



in neutral to slightly alkaline solutions, a common practice is to estimate these rates by extrapolation of the rate of alkaline hydrolysis to the pH at which the catalyst operates. This estimate involves the assumption that hydrolysis depends linearly on hydroxide concentration for a wide pH range. For triesters,[38a] diesters,[31] and monoesters[39] of phosphoric acid, pH–rate profiles are complex and this assumption is questionable. These discrepancies are evident if one calculates the rate enhancements for the $Cu(bpy)^{2+}$-catalyzed hydrolysis of **1** and **2** from the data in Table I. At 75 °C K_w is 2.0×10^{-13} M. Extrapolation of the rate of hydrolysis by NaOH to pH 6.5, assuming a first-order hydroxide dependence, yields an enhancement of 68 000 and 7000 for **1** and **2**, respectively. Comparison of the catalyzed reaction rate to the directly measured rate of hydrolysis from a control experiment at a pH of 6.5 gives an enhancement of 2000 and 150 for **1** and **2**, respectively. The greater than 30-fold difference in these enhancements arises from the neglected contribution of the pH-independent hydrolysis and from a hydroxide dependence that is less than 1.[31a] The former values may be more relevant in mechanistic comparisons of reactions with metal ion catalysis to reactions with hydroxide as a nucleophile if the alkaline hydrolysis is first order in hydroxide; however, the latter values are more pertinent from a practical standpoint. The third and most rigorous method of calculating rate enhancements of phosphate ester hydrolysis catalyzed by metal complexes involves comparison of the rate of hydrolysis of the bound phosphate ester to that of hydrolysis without catalyst. A rate enhancement of 6300 is calculated in this manner for **2** bound to $Cu(bpy)^{2+}$ by using the rate constant for decomposition of Cu(bpy)(OH)(**2**) to products (k_{cat}; Scheme I). Although these rate constants are pertinent from a mechanistic standpoint, they may be misleading; hydrolysis of **1** by $Cu(bpy)^{2+}$ (1 mM) proceeds faster than hydrolysis of **1** by $Co(en)_2(OH)(OH_2)^{2+}$ (1 mM; en = ethylenediamine) at pH 7 and 50 °C, yet a rate enhancement of 10^7 was recently reported for the latter hydrolysis.[40] Thus, in comparing literature data on rate enhancements, one must carefully examine the method of analysis.

Ideal catalyst behavior is observed for greater than 1000 turnovers in the hydrolysis of **1** whereas the rate of hydrolysis of **2** slowly begins to decrease after ~50 turnovers. Loss of activity does not result from product inhibition by ethyl phosphate and is not affected by the presence of oxygen. Although we do not yet understand the basis for catalyst poisoning in hydrolysis of the aryl alkyl phosphate ester, this problem emphasizes the importance of examining catalyst longevity. Production of slightly greater than stoichiometric amounts of product, or evidence for a few turnovers, is not adequate characterization of a catalytic system.

Mechanism of Diester Hydrolysis. The mechanism proposed in Schemes I and II accords with the pH–rate profile, a first-order dependence on $Cu(bpy)^{2+}$ and phosphate ester at low concentrations ($<1 \times 10^{-4}$ M and <0.005 M, respectively), saturation kinetics in **2** at high ratios of **2** to $Cu(bpy)^{2+}$, and the ^{18}O-labeling studies. In these schemes only the mechanism of hydrolysis at alkaline pH is shown. A five-coordinate phosphorane intermediate is proposed in analogy to the mechanism of base hydrolysis of organic phosphate esters; however, a concerted mechanism may be operative (Scheme II). A sigmoidal pH–rate profile suggests that the removal of a proton with a pK_a of 7.0 is necessary to form the dominant catalyst at pH >6.3. The similarity between the kinetic pK_a and that measured for $Cu(bpy)^{2+}$ (7.1)[35] suggests formation of a copper-bound hydroxide or its kinetic equivalent although the competing dimerization of $Cu(bpy)(OH)^+$ makes this value somewhat uncertain.[33] The generality of this type of pH–rate profile is suggested by a matching pH dependence observed in the hydrolysis of phosphate triesters[37b] and dialkyl fluorophosphates[9a] by $Cu(bpy)^{2+}$. Evidence for copper hydroxides as the active catalysts in the hydrolysis of phosphorus esters has been reported for fluorophosphonates,[9b] phosphate triesters,[15] and ATP.[10] The pH-independent hydrolysis, which dominates at pH <6.3, could occur by attack of a coordinated water molecule of the copper complex on **2** in analogy to the proposed Cu–OH pathway; however, we have no kinetic evidence to rule out intermolecular hydrolysis.

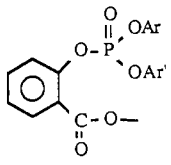
Saturation kinetics with respect to **2** at high ratios of **2** to catalyst provide evidence for an intermediate copper(II) phosphate ester complex. Since Cu(II) complexes exchange aquo and anionic ligands rapidly,[41] compared to the rate of hydrolysis observed here, the rate-limiting step must involve breakdown of the copper(II) phosphate ester complex into products. If the exchange of **2** as a ligand occurs much faster than hydrolysis ($k_d \gg 5.60 \times 10^{-4}$ s^{-1}), then K_{eq} for coordination of **2** is ~20 M^{-1}. This low value[42] confirms the expectation that phosphate diesters are weak ligands toward Cu(II) as compared to amines[43] and explains why even bulky amines, such as the buffers, inhibit catalysis.

A reasonable formulation for the copper(II) hydroxide–phosphate ester intermediate (**5**) of Scheme II has hydroxy and phosphate ester ligands bound cis to $Cu(bpy)^{2+}$. The copper(II) complex plays a bifunctional role effecting hydrolysis by electrophilic activation of the coordinated phosphate ester and by providing a cis proximity for the hydroxide nucleophile. In support of this mechanism we observe that copper(II) complexes with one less available coordination site, such as $Cu(terpyridine)^{2+}$ or $Cu(diethylenetriamine)^{2+}$, do not promote hydrolysis of phosphate diesters **1** or **2**, respectively. Kinetically equivalent mechanisms must incorporate the dual features of phosphate diester complexation and evidence for a Cu–OH species as active catalyst. Intramolecular reactions can show extremely large rate enhancements[44] from template effects and the gain in translational entropy over that for an intermolecular process. On the basis of pH–rate profiles and the magnitude of the rate enhancements, intramolecular base hydrolysis of phosphate esters has been

(38) (a) Khan, S. A.; Kirby, A. J. *J. Chem. Soc. B* **1970**, 1172–1182. (b) Kirby, A. J.; Varboglis, A. G. *J. Am. Chem. Soc.* **1967**, *89*, 415. (c) See ref 46.
(39) (a) Bailey, M. C. *Bull. Soc. Chim. Fr. Mem.* **1942**, *9*, 314. (b) Kirby, A. J.; Varboglis, A. G. *J. Am. Chem. Soc.* **1967**, *89*, 415. (c) Butcher, W.; Westheimer, F. H. *J. Am. Chem. Soc.* **1955**, *77*, 2420. (d) Kirby, A. J.; Jencks, W. P. *J. Am. Chem. Soc.* **1965**, *87*, 3209–3216. (e) Kumamoto, J.; Westheimer, F. H. *J. Am. Chem. Soc.* **1955**, *77*, 2515–2518.
(40) (a) k_{obs} at 50 °C, 1 mM $Cu(bpy)^{2+}$, and pH 7.0 is 3.5×10^{-6} s^{-1}. (b) Chin, J. K.; Xiang, Z. *J. Am. Chem. Soc.* **1988**, *110*, 223–225.
(41) Frey, C. M.; Stuehr, J. E. *Metal Ions in Biological Systems*; Dekker: New York, 1974; Vol. 1, pp 51–116.
(42) A reviewer has commented on the magnitude of this equilibrium constant in comparison to the reported binding constant of $H_2PO_4^{2-}$ and $Co(NH_3)_5OH_2^{3+}$ of 8 (see: Schmidt, W.; Taube, H. *Inorg. Chem.* **1963**, *2*, 698). However, the anomalously high magnitude of Cu(II) binding constants within the first-row transition-metal series is documented for other phosphate ligands,[53] and the Co^{3+} complex considered by the reviewer is an outer-sphere complex.
(43) *Critical Stability Constants*; Martell, A. E., Smith, R. M., Eds.; Plenum: New York, 1974; Vol. 2.
(44) Menger, F. M. *Acc. Chem. Res.* **1985**, *18*, 128.

proposed for Cu(II),[15] Co(III),[6,45] and Zn(II)[8] complexes. Stoichiometric base hydrolysis of $(en)_2Co(^{18}OH_2)[(4\text{-}NO_2C_6H_4O)PO_3]$ to yield ^{18}O-labeled coordinated inorganic phosphate provides the strongest evidence for intramolecular hydrolysis of phosphate esters by transition-metal complexes.[6a] Since $Cu(bpy)^{2+}$, unlike Co(III), exchanges aquo ligands rapidly on the time scale of hydrolysis, no mechanistic discrimination between inter- and intramolecular hydrolyses is possible by labeling experiments here.

Unless a concerted mechanism operates, hydrolysis of $Cu(bpy)(OH)((4\text{-}NO_2C_6H_4O)(CH_3CH_2O)PO_2)$ (**5**) could proceed through a five-coordinate phosphorane, as has been proposed for hydrolysis of cyclic phosphate esters.[46] Intramolecular nucleophilic attack is identified as the dominant reaction pathway in the hydrolysis of **6**[47] and **7**,[48] although intramolecular general



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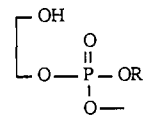

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acid/base catalysis occurs for other phosphate esters, where neighboring group participation accelerates hydrolysis.[16] Since phosphoranes are stabilized when they are part of a ring or when they have pendant electronegative groups such as oxygen ligands,[46a] formation of a phosphorane intermediate in **5**–**7** by intramolecular attack should be favorable. Stabilization of a phosphorane intermediate by the presence of a five-membered ring has been proposed[49] to account for the rapid water exchange and ring hydrolysis in ethylene hydrogen phosphate. Similarly, the catalytic effect of transition-metal complexes on the hydrolysis of phosphate esters has been attributed to stabilization of a phosphorane intermediate by complexation.[5,12] Labeling experiments[6a] and product distribution studies[8] support the existence of a metallacyclic phosphorane complex as an intermediate in the hydrolysis of phosphate esters. Labeling studies here, which show neither double-label incorporation into monoester product nor any incorporation of label into diester substrate, do not provide direct evidence for an intermediate phosphorane species.

The exclusive hydrolysis of *p*-nitrophenol from **2** by $Cu(bpy)^{2+}$ correlates with the large pK_a difference between *p*-nitrophenol and ethanol. Similarly, **3** is hydrolyzed by $Cu(bpy)^{2+}$ but ethyl phosphate is not. These results are in harmony with nucleophilic displacement reactions at phosphorus in phosphate diesters[38a] and phosphate monoesters[38b] (not involving metals), where a strong dependence on leaving group pK_a is observed. To a lesser extent, the rate of nucleophilic displacement at phosphorus in phosphate esters depends on the substituents of the group not displaced.[38c] Although the rate constant for hydrolysis of **1** was not broken down into a binding constant and a rate constant for hydrolysis, it is not likely that **1** binds better than **2**; therefore, the more rapid $Cu(bpy)^{2+}$-catalyzed hydrolysis of **1** compared to that of **2** may be attributed to the favorable effect in **1** of a second 4-nitrophenyl group, which makes the phosphorus center more electrophilic and susceptible to hydrolysis.

Monoester and Triester Hydrolysis. Hydrolysis of the phosphate triester **4** by $Cu(bpy)^{2+}$ resembles that of **2** in the observed first-order dependences on phosphate ester and $Cu(bpy)^{2+}$ concentrations, as well as in the pH–rate profile.[37b] However, hydrolysis of **4** occurs more than 30 times faster than hydrolysis of **2** on the alkaline pH plateau (Table VI). Although the equilibrium constant for formation of a triester complex should be lower for the sterically bulky and neutral triester than for the anionic diester, enhanced intramolecular attack on the more positive phosphorus center may compensate. Phosphate triesters are usually more reactive than phosphate diesters toward intramolecular nucleophilic attack of oxyanions.[50]

Hydrolysis of the dianion of **3** by $Cu(bpy)^{2+}$ occurs as the slowest of all the phosphate ester hydrolyses under alkaline conditions. The order of observed rates triester > diester (anion) > monoester (dianion) parallels that for addition of anionic nucleophiles to phosphate esters. However, the magnitude of the difference in reactivity between the triester and diester toward monoanionic nucleophiles can be greater than 10^3 and approaches 10^{10} for methyl esters;[19] a factor of 10^2 is attributed to electrostatic repulsion.[17a] The monoester dianion does not react with anionic nucleophiles under these conditions. The greatly mitigated reactivity differences in the $Cu(bpy)^{2+}$-catalyzed hydrolysis of the three esters may reflect a reduced electrostatic barrier as a result of electrophilic activation of the coordinated ester, the template effect of positioning nucleophile and phosphate ester in close proximity, and the better binding of monoesters over diesters over triesters. Since we did not observe saturation kinetics for all three esters, we cannot directly compare true second-order rate constants for the three catalyst–substrate complexes.

Both the observed order of phosphate ester reactivity, which does not depart from that expected for nucleophilic oxyanion attack, and the positive rate enhancements suggests that the dianion of **3** is hydrolyzed by a reaction pathway similar to that presented for the phosphate diester in Scheme I and not by a dissociative (metaphosphate) pathway. This mechanism resembles that for the hydrolysis of the dianion **3** bound to Co(III), although much larger rate enhancements (10^7) are observed for the cobalt complexes.[6a] In contrast, a dissociative transition state has been proposed for hydrolysis of the dianions of phosphate monoesters in the presence of Mg^{2+} and Ca^{2+}; these metal cations inhibit phosphate ester hydrolysis.[51]

Other Metal Complexes. Phosphate diester hydrolysis should be favored by a large formation constant for metal complex and ester (Scheme I). For divalent metal ions, formation constants for ligands with oxygen donor atoms[52] do not vary as dramatically as do those for ligands with nitrogen donor atoms (K_f (25 °C) for oxalate: Mg^{2+}, 2.7×10^3 M^{-1}; Ni^{2+}, 1.4×10^5 M^{-1}; Cu^{2+}, 1.7×10^6 M^{-1}. K_f (25 °C) for ethylenediamine: Mg^{2+}, 2.3 M^{-1}; Ni^{2+}, 3.0×10^7 M^{-1}; Cu^{2+}, 5.1×10^{10} M^{-1}).[43] Similarly, formation constants for hydrogen phosphate (HPO_4^{2-}) show relatively small variations (K_f (25 °C): Mg^{2+}, 5.0×10^1 M^{-1}; Ni^{2+}, 1.2×10^2 M^{-1}; Cu^{2+}, 1.6×10^3 M^{-1}).[43,53] If one assumes a similar trend for phosphate diester complexation, the effectiveness of Cu(II) as a catalyst in the series of aquo complexes cannot be explained simply by degree of complex formation alone. The reason for effective hydrolysis of phosphate diesters by Cu^{2+} probably results from the low pK_a of water bound to Cu^{2+}; none of the other metal aquo complexes are deprotonated to a significant extent at neutral pH. As observed for $Cu(bpy)^{2+}$ vs $Cu(bpy)(OH)^+$ hydrolysis of **2**, hydrolysis by a metal hydroxide complex occurs faster than hydrolysis by a metal aquo complex.

The beneficial effect of a neutral amine chelate on the hydrolysis of **1** or **2** by Cu^{2+} or Ni^{2+} cations is evident from hydrolysis rates observed for $Cu(bpy)^{2+}$ and $Ni(tren)^{2+}$ (Table I). For nickel(II)-promoted hydrolysis of phosphate esters the tren ligand was necessary to obtain catalytic turnover as well as higher rates of

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hydrolysis. Of the several simple amines that were tried with copper(II), 2,2′-bipyridine gave the most satisfactory combination of high hydrolysis rates and catalyst longevity. Bipyridine complexes of Cu(II) may be better catalysts for phosphate ester hydrolysis because of a favorable change in the equilibrium constant for coordination to anionic ligands promoted by the π acidity of 2,2′-bipyridine[54] or by some favorable effect in the decomposition of **5** to products.

Conclusions

These studies show that one can develop long-lived transition-metal catalysts for the hydrolysis of phosphate diesters in aqueous solution near neutral pH. The mechanism of catalysis includes substrate binding to the metal hydroxide complex, followed by intramolecular attack of hydroxide on phosphate ester, and P–O bond cleavage. The cationic metal catalyst effectively neutralizes the electrostatic barrier to hydroxide nucleophilic attack on phosphate diesters and monoesters; these anionic esters are hydrolyzed only 60-fold more slowly than the neutral phosphate triester. Of the first-row divalent transition-metal cations surveyed, Ni^{2+} and Cu^{2+} having high catalytic activity may reflect a favorable binding that occurs reversibly for these softer metals and a low pK_a for coordinated water. Metal-ion-catalyzed hydrolysis of phosphate diesters could play a role in DNA damage by transition-metal ions, as well as in the development of footprinting reagents. Further studies along these lines are under way.

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Supplementary Material Available: Tables of kinetic data for hydrolysis of phosphate esters by $Cu(bpy)^{2+}$ (6 pages). Ordering information is given on any current masthead page.

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Dimeric Complexes Containing the $[Fe_2S_2]^{2+}$ Cores Coordinated by Non-Sulfur Terminal Ligands.[1] Synthesis, Structural Characterization, and Spectroscopic Properties of $[Et_4N]_2[Fe_2S_2(o,o'\text{-}C_{12}H_8O_2)_2]$, $[Et_4N]_2[Fe_2S_2(C_4H_4N)_4]$, and $[Et_4N]_2[Fe_2S_2(O\text{-}o\text{-}C_6H_4CH(n\text{-}C_4H_9)NHC_6H_4\text{-}o\text{-}S)_2]$ and the Structure of $[Ph_4P]_2[Fe_2S_2(OC_6H_4\text{-}p\text{-}CH_3)_4]$

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The synthesis and spectroscopic characterization of the $[Fe_2S_2L_4]^{2-}$ clusters (L_2 = $o,o'\text{-}C_{12}H_8O_2^{2-}$, $^-O\text{-}o\text{-}C_6H_4CH(n\text{-}Bu)NHC_6H_4\text{-}o\text{-}S^-$; L = $^-OC_6H_4\text{-}p\text{-}CH_3$, $C_4H_4N^-$) are reported. The crystal and molecular structures of $(Et_4N)_2[Fe_2S_2(o,o'\text{-}C_{12}H_8O_2)_2]$ (I), $(Et_4N)_2[Fe_2S_2(C_4H_4N)_4]$ (II), $(Et_4N)_2[Fe_2S_2(O\text{-}o\text{-}C_6H_4CH(n\text{-}Bu)NHC_6H_4\text{-}o\text{-}S)_2]$ (III), and $(Ph_4P)_2[Fe_2S_2(OC_6H_4\text{-}p\text{-}CH_3)_4]$ (IV) are described in detail. I crystallizes in the monoclinic space group $C2/c$, with cell dimensions a = 11.500 (2) Å, b = 13.541 (3) Å, c = 26.612 (5) Å, β = 92.25 (1)°, and Z = 4. II crystallizes in monoclinic space group $P2_1/n$, with cell dimensions a = 9.689 (3) Å, b = 16.362 (2) Å, c = 11.910 (5) Å, β = 97.74 (3)°, and Z = 2. III crystallizes in the monoclinic space group $P2_1/c$, with cell dimensions a = 14.878 (4) Å, b = 9.585 (3) Å, c = 18.950 (6) Å, β = 95.59 (2)°, and Z = 2, and IV crystallizes in the monoclinic space group $P2_1/a$, with cell dimensions a = 16.308 (5) Å, b = 16.674 (6) Å, c = 24.456 (9) Å, β = 91.13 (3)°, and Z = 4. Intensity data for I–IV were collected on a four-circle computer-controlled diffractometer with use of the θ–2θ scan technique. All four structures were solved by conventional methods from 2739, 1736, 2086 and 3632 reflections for I–IV, respectively. The structures were refined by full-matrix least-squares techniques (310 parameters for I, 173 parameters for II, 280 parameters for III, and 385 parameters for IV) to final R values of 0.033 (I), 0.069 (II), 0.065 (III), and 0.074 (IV). Complexes I–IV contain the planar $[Fe_2S_2]^{2+}$ cores coordinated fully or partially by non-sulfur terminal ligands. The Fe···Fe distance in the centrosymmetric dianions ranges from 2.677 (3) Å in II to 2.749 (24) Å in IV. The Fe–O distances are 1.894 (2) (I), 1.921 (11) (III), and 1.870 (12) Å (IV), and the $\overline{Fe\text{–}N}$ distance in II is 1.97 (5) Å. Structural comparisons with the already reported thiolate dimers $[Fe_2S_2L_4]^{2-}$ (L = ^-SAr; L_2 = $S_2\text{-}o\text{-}xyl^{2-}$) are reported. The electronic, Mössbauer, cyclic voltammetric, and proton magnetic resonance properties of the new dimers are discussed in detail. A comparison of these properties with the available data on the Rieske $[Fe_2S_2]$ centers is presented and certain conclusions are drawn concerning future design of synthetic analogues.

Introduction

A detailed understanding of the metal centers in the plant type two iron ferredoxins[4] was greatly facilitated by the isolation and structural characterization of several synthetic analogue complexes with the general formula $[L_2FeS_2FeL_2]^{2-}$ (L_2 = o-xylene-α,α'-dithiolate;[5] L = $^-S\text{-}C_6H_4\text{-}p\text{-}CH_3$,[5] Cl^-,[5b] L_2 = S_5^{2-}[6]). Detailed comparative studies of the Mossbauer, electron paramagnetic resonance (EPR), and electronic spectra of the dimeric analogue complexes[7] and of representative 2Fe-2S ferredoxins led[8] to the realization that the iron–sulfur cores in the latter were structurally identical with the planar rhombic Fe_2S_2 units in the former. The

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