

Notes

Catalytic Effects of Cu(II)-TMED and Cu(II)-BIPY on the Hydrolysis of *p*-Nitrophenyl Diphenyl Phosphate

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Phosphate esters and related phosphorus(V) materials have been employed for chemical warfare, pest control, and numerous industrial tasks. And most of the phosphates have strong toxicity for human body and animals. So the fast decomposition of toxic P(V) compounds to nontoxic materials is useful in the view point of environmental protection. It has been well known that metal complexes are capable of acting as good hydrolytic catalysts for toxic fluorophosphates.¹⁻⁵ But there are very few reports concerning phosphate triester such as PNPDP (*p*-nitrophenyl diphenylphosphate) by homogeneous Cu(II)-chelates. PNDPP was selected as the principal substrate for our study because: (a) It is an easily handled simulant of very dangerous phosphorous(V) compounds such as Soman (pinacolyl methyl phosphoponefluoridate), (b) The hydrolysis of PNPDP can be easily monitored spectrophotometrically. The purpose of this investigation was to study the catalytic effects of Cu(II)-TMED and Cu(II)-BIPY chelates on the hydrolysis of the PNPDP.

Experimental

Materials. *p*-Nitrophenyl diphenylphosphate was prepared as described,⁶ mp. 47-48°C [lit. mp. 49-51°C]. HEPES (N-[2-hydroxyethyl]-piperazine-N'-ethanesulfonic acid), was purchased from Sigma. N,N,N',N'-tetramethylethylenediamine (TMED), and 2,2'-bipyridine(BIPY), reagent grade, were purchased from Aldrich Chemicals. CuCl₂·2H₂O was purchased from Fisher Scientific, and dehydrated in a vacuum-dry oven.

Preparation of Cu(II)-Chelates. Cu(BIPY)Cl₂ and Cu(TMED)Cl₂ complexes were prepared by the following general method. A solution of 15 mmoles of the diamine ligand in 30 ml of absolute ethanol was added slowly with magnetic stirring to a solution of 15 mmoles of anhydrous CuCl₂ in 30 ml of ethanol. The resulting precipitate was washed with two portions of cold absolute ethanol (30 ml each), recrystallized from absolute ethanol or ethanol-ether, and dried in a vacuum desiccator for 5 hours. Cu(TMED)Cl₂: mp. 134-5°C. Cu(BIPY)Cl₂: mp. 330-3°C (decom).

Kinetics. The following kinetic run is typical of that used throughout the experiment. 1-cm cuvette was filled with 20 mM HEPES buffer (2.0 ml, I=0.1 M, NaCl, pH 8.0) containing 0.5 mM Cu(II)-BIPY or Cu(II)-TMED. The cuvette was then stoppered and placed for 15 min in the sample chamber of a Hewlett Packard 8452A Diode Array spectro-

Table 1. Pseudo-First-Order Rate Constants of the Hydrolysis of PNPDP by Catalysts^a

Catalysts	Conc.	k_{obs} (sec ⁻¹)
Cu(II)-TMED	0.10 mM	$(8.58 \pm 0.36) \times 10^{-5}$
	0.20 mM	$(1.92 \pm 0.04) \times 10^{-4}$
	0.24 mM	$(2.35 \pm 0.02) \times 10^{-4}$
	0.28 mM	$(3.24 \pm 0.30) \times 10^{-4}$
	0.40 mM	$(4.75 \pm 0.09) \times 10^{-4}$
	0.50 mM	$(6.03 \pm 0.17) \times 10^{-4}$
Cu(II)-BIPY	0.10 mM	$(9.04 \pm 0.07) \times 10^{-5}$
	0.20 mM	$(1.72 \pm 0.05) \times 10^{-4}$
	0.50 mM	$(3.28 \pm 0.09) \times 10^{-4}$
	0.70 mM	$(4.15 \pm 0.13) \times 10^{-4}$
	1.00 mM	$(6.28 \pm 0.08) \times 10^{-4}$
HEPES buffer NaOH ^b	20.0 mM	$(9.29 \pm 0.03) \times 10^{-6}$
	2.00 mM	$(6.51 \pm 0.03) \times 10^{-4}$
	5.00 mM	$(2.10 \pm 0.50) \times 10^{-3}$
	10.0 mM	$(4.11 \pm 0.12) \times 10^{-3}$
	20.0 mM	$(7.71 \pm 0.20) \times 10^{-3}$

^aAll reactions were carried out in 20 mM HEPES buffer, pH 8.0, 4% acetonitrile (v/v) unless otherwise indicated. ^bNot at pH 8.0.

photometer thermostated at 25±0.1°C. The reaction was initiated by adding acetonitrile (80 μl) containing 0.25 mM PNPDP followed by constant stirring of the solution with a magnetic bar contained in the cuvette. Liberation of *p*-nitrophenolate as a reaction product was monitored at 400 nm for more than 6 half-lives. Pseudo-first-order rate constants were calculated by using Hewlett Packard kinetic software package to the following equation: $\ln(A_{\infty} - A_t) = kt + \ln(A_{\infty} - A_0)$. All experiments were run in duplicate or triplicate, and tabulated data represent the average of these experiments. Rate constants were reproducible to ±10%. Separately, blank experiments containing PNDPP and HEPES buffer (pH 8.0 and I=0.10 M) were run. Because PNPDP is insoluble in pure water, we selected CH₃CN as a cosolvent that would allow sufficient solubilities of catalysts and substrate. Most of these experiments were performed in HEPES buffer solution containing 4% (v/v) of CH₃CN.

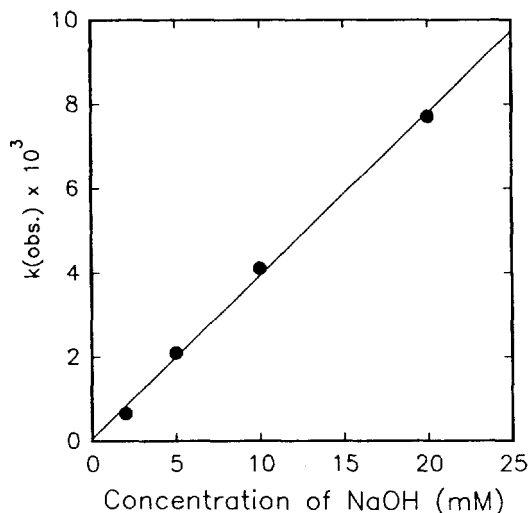
Results and Discussion

Representative pseudo-first order rate constants are demonstrated in Table 1. A K_{obs} of $9.29 \times 10^{-6} \text{ s}^{-1}$ at pH 8.00, 20 mM HEPES, I=0.100 M, was obtained for the hydrolysis of PNPDP in the absence of copper(II)-chelates. Both copper(II)-chelates promoted the hydrolysis of PNPDP significantly. The reaction in the presence of 0.5 mM Cu(II)-TMED proceeds 65 times faster than the one in the absence of the Cu(II)-chelates at pH 8.0. Hydrolysis of PNPDP by two Cu(II)-chelates showed a first order dependence on the concentration of metal complex. Apparent-second-order rate con-

Table 2. Apparent Second-Order Rate Constants for PNPDP at pH 8.00^a

Hydrolytic agents	app k_2 , $M^{-1}s^{-1b}$
Cu(II)-TMED	1.32
Cu(II)-BIPY	5.7×10^{-1}
NaOH ^c	3.9×10^{-1}

^a0.01 mM initial PNPDP concentration. ^bLinear regression analyses of pseudo-first-order rate constants show correlation coefficients >0.995. ^cNot at pH 8.0

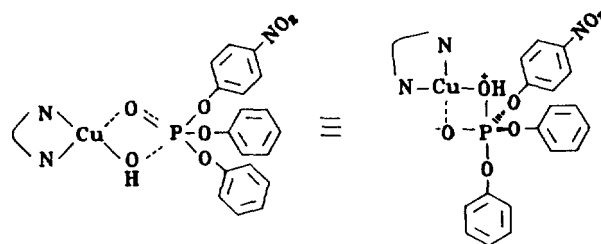
**Figure 1.** Dependence of rate constants on the concentration of NaOH for the hydrolysis of PNPDP.

stants (Table 2) were determined from plots of pseudo-first-order rate constants (Table 1) versus Cu(II)-TMED and Cu(II)-BIPY concentration. It was found that Cu(II)-TMED is more effective than Cu(II)-BIPY. The second-order-rate constants of Cu(II)-TMED and Cu(II)-BIPY were estimated to be $1.32 M^{-1}s^{-1}$ and $5.7 \times 10^{-1} M^{-1}s^{-1}$ respectively.

The alkaline hydrolyses of PNPDP are demonstrated in Figure 1. Hydrolysis of PNPDP by sodium hydroxide showed a first order dependence on the concentration of sodium hydroxide. Approximately the calculated second-order-rate constant for the hydrolysis of PNPDP by hydroxide was $3.9 \times 10^{-1} M^{-1}s^{-1}$. It is well known that metal ion can lower the pK_a value of a ligated water molecule, generating relatively larger concentration of a nucleophilic catalyst, the metal bound hydroxide, near neutral pH.^{7,8} Evidence for copper hydroxides as the active catalysts in the hydrolysis of phosphorus esters has been reported for fluorophosphonates,⁴ phosphate triesters,⁷ and ATP.⁹ Considering these data, we suppose that two species could conceivably function as the catalytically active nucleophile: (a) copper-bound hydroxide formed by dissociation of a proton on a water molecule ligated to the metal center of copper(II)-chelates (Eq. (1)) and (b) free hydroxide ion in the solution.

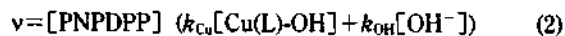


Since free hydroxide ion is undoubtedly more basic than Cu(L)-OH, some remarkable polarizability effects would be

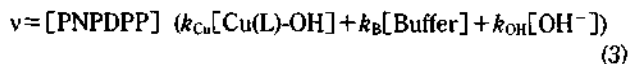
**Scheme 1.**

required to make Cu(L)-OH a good nucleophile. Therefore, it is supposed that push-pull or bifunctional mechanism would be suitable for a catalytic species involving copper(II)-chelates. The copper center delivers a coordinated hydroxide nucleophilically to the phosphorous atom, while simultaneously drawing electron density away from the phosphorus atom by interacting with the phosphoryl oxygen (Scheme 1).

Similar bifunctional mechanism has been invoked for metal chelate mediated hydrolysis of isopropylmethyl phosphonofluoridate.^{4,5,10} In these cases, monohydroxy-metal complexes were reported to be implicated as active species at mildly alkaline pH, and the apparent second-order-rate constants for reactions of the complexes with substrate were observed to be higher than the one for the reaction of hydroxide with substrate. Since the reaction of Cu(L)-OH with the substrate shows second-order kinetics, we can describe the hydrolysis rate of PNPDP as the sum of two second-order terms, as shown in Eq. (2).



k_{Cu} and k_{OH} are, respectively, second-order rate constants for hydrolytic processes mediated by Cu(L)-OH and hydroxide. Since k_{OH} is $3.9 \times 10^{-1} M^{-1}s^{-1}$ $k_{OH}[OH^-]$ equals to $3.9 \times 10^{-7} s^{-1}$ at pH 8.0 in our system. However, k_{obs} for the blank test ($9.29 \times 10^{-6} s^{-1}$) was greater than $k_{OH}[OH^-]$ in Eq. (2). We may regard that HEPES buffer could catalyze the hydrolysis of PNPDP and mainly attribute to the k_{obs} for the blank test. Ward and Albizo¹¹ measured HEPES-catalyzed hydrolysis of Soman and determined a value of $1.6 \times 10^{-3} M^{-1}s^{-1}$ for k_{buffer} . Therefore we could add $k_B[Buffer]$ term to Eq. (2) and reduce to Eq. (3).



$k_B[Buffer]$ and $k_{OH}[OH^-]$ values are very small compared to the observed rate constants ($\sim 10^{-4} s^{-1}$), we can reduce Eq. (3) to Eq. (4).



On the basis of the above considerations, we suggest that two Cu(II)-chelates may act as catalyst in the hydrolysis of PNPDP. The result that the rate of hydrolysis of PNPDP by Cu(II)-TMED was greater than Cu(II)-BIPY might be explained by electronic effect of two ligands which was suggested by Martell.¹² It would perhaps be expected that in a chelate compound in which the copper(II) ion forms weaker bonds with a ligand, the metal would have greater affinity for an additional donor group such as a hydroxyl ion. The value of the acid dissociation constant of BIPY(sp_2 hybrid) is smaller than that of TMED(sp_3 hybrid). Therefore, the

monohydroxy chelate of BIPY, which is less basic than TMED, has greater affinity for an additional donor atom than is the analogous chelates of the aliphatic diamines; hence dimer formation takes place more easily in the case of the aromatic compounds. The dimers of these chelate compounds were known to possess negligible catalytic activity.^{5,12}

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Catalysis of Triamine-copper(II)-imidazolate Complexes for Ester Hydrolysis

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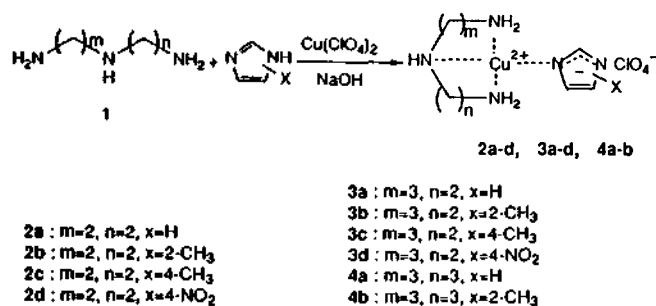
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Imidazole and imidazolate as catalysts for ester hydrolyses have aroused many worker's interest,¹ since they have an essential active site of several hydrolytic enzymes.² Recently imidazole when co-ordinated to a copper(II) complex of diethylenetriamine (2a) was reported to exhibit a marked increase in catalytic effect on the hydrolysis of *p*-nitrophenyl benzoate.³

In this work, we have synthesized nine additional deriva-



Scheme 1.

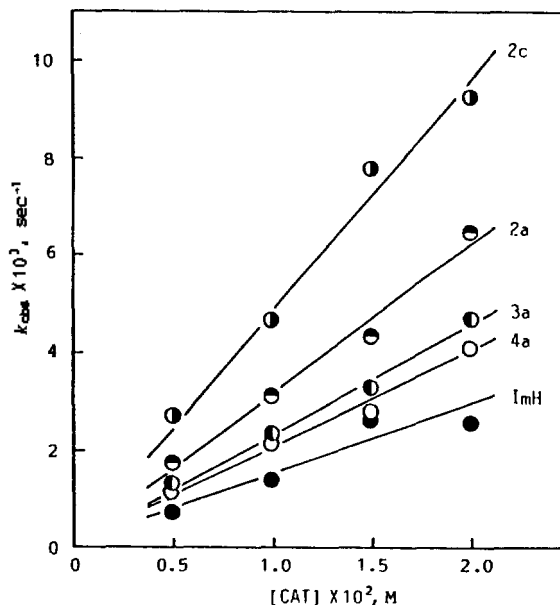


Figure 1. Plots of [CAT] vs. k_{obs} for the hydrolysis of *p*-nitrophenyl acetate at 25°C (pH 7.0).

tives of triamine-copper(II)-imidazolate complexes (2b-4b in Scheme 1), and examined their catalytic activity to the hydrolysis of *p*-nitrophenyl acetate (NPA).

Catalytic actions of these complexes to the hydrolysis of *p*-nitrophenyl acetate, $\text{PNA} + \text{H}_2\text{O} \xrightarrow{\text{CAT}} \text{AcOH} + \text{p-nitrophenol}$, were investigated by a similar method utilized for the imidazole catalyzed hydrolysis of the same substrate by Lombardo.⁴ The rate law applied to this study is written in Eqs. (1)-(3), where k_o is the first-order rate constant for the uncatalyzed hydrolysis and k_{cat} is the second-order rate constant for the catalysis. Under pseudo first-order conditions in which $[\text{CAT}] \gg [\text{NPA}]$, Eq. (1) is reduced to Eq. (2) and Eq. (3). As shown several examples in Figure 1, plots of k_{obs} against $[\text{CAT}]$ revealed straight lines. Then k_{cat} values were derived (Table 1) from slopes of the lines.

$$\text{rate} = (k_o + k_{cat}[\text{CAT}]^n) [\text{NPA}] \quad (1)$$

$$\text{rate} = k_{obs}[\text{NPA}] \quad (2)$$

$$k_{obs} = k_o + k_{cat}[\text{CAT}]^n \quad (3)$$

The first-order rate constants (k_o) for the uncatalyzed hydrolysis, obtained from intercepts of lines in Figure 1, are in the range of $4.95\text{--}5.25 \times 10^{-5} \text{ s}^{-1}$. The ring size of complexes seems to affect the catalytic activity. The complex consists