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Multifunctional Catalysis by Metal Complexes

1. Ester Hydrolysis Catalyzed by Oximinatozinc(II) Ions

JUNGHUN SUH,[1] MINSEK CHEONG, AND HYUNSOO HAN

Department of Chemistry, Seoul National University, Seoul 151, Korea

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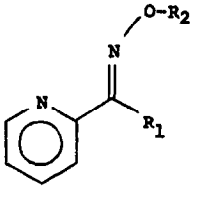
Hydrolysis of *p*-nitrophenyl acetate catalyzed by a Zn(II) complex of 2-acetylpyridineketoxime or 2-pyridinecarboxaldoxime was studied as a model of multifunctional catalysis by metalloproteases. The reaction proceeded exclusively through the formation of an acyl–catalyst intermediate under the experimental conditions, and both the formation and the breakdown of the acyl intermediate were much faster than the spontaneous reaction. The metal ion, the metal-bound water molecule or hydroxide ion, the oximate ion, and general bases contributed to the multifunctional catalysis in ester hydrolysis by the oximinatozinc(II) ions.

To date, mechanistic studies on the metal-catalyzed hydrolysis of acyl derivatives have usually employed metal ions as the sole catalyst (*1*). When metal complexes are employed, the acylation of the ligand, instead of the hydrolysis of the acyl substrate, has been the major subject (*2–7*). Metal ions in enzymatic systems, however, act together with several other active-site functional groups, leading to highly cooperative catalytic effects. Therefore, multifunctional catalysis by a metal ion and the functional groups of the ligand would be a more advanced model of metalloenzymes. In view of the efficient hydrolysis of *O*-acetyl-2-acetylpyridineketoxime (**A**) and *O*-acetyl-2-pyridinecarboxaldoxime (**B**) in the presence of metal ions (*8–10*), we have extended the study to the catalysis by the Zn(II) complex of 2-acetylpyridineketoxime (**C**) or 2-pyridinecarboxaldoxime (**D**) in the hydrolysis of *p*-nitrophenyl acetate (PNPA).



A: $R_1 = CH_3$, $R_2 = CH_3CO$

B: $R_1 = H$, $R_2 = CH_3CO$

C: $R_1 = CH_3$, $R_2 = H$

D: $R_1 = H$, $R_2 = H$

[1] To whom inquiries should be addressed.

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EXPERIMENTAL PROCEDURES

Materials. Compound **C** was prepared according to the literature (*11*), mp 120–121°C (Ref. (*11*), 121°C). Preparation of **D** was described previously (*9*). PNPA was the commercial product (Aldrich) and was used after recrystallization from ether. Solutions of cupric chloride and zinc chloride were prepared by dissolving cupric oxide and zinc oxide (both from Aldrich, "Gold Label"), respectively, with hydrochloric acid. Acetonitrile was purified according to the literature (*12*). Water was distilled and deionized before use in kinetic studies.

Kinetic measurements. Reaction rates were measured with a Beckman 5260 uv/vis spectrophotometer. Temperature was controlled to within ±0.1°C with a Haake E52 circulator. pH measurements were carried out with a Fisher Accumet 525 pH meter. Kinetics were performed at an ionic strength of 1.0, which was adjusted with sodium chloride. Mes (4-morpholineethanesulfonic acid) was used as the buffer. The stock solution of PNPA was made in acetonitrile and the reaction mixtures for kinetic studies contained 0.8 %(v/v) acetonitrile. Acetylation of **C** with PNPA in the presence of Zn^{2+} was investigated with 0.001–0.1 *M* Zn^{2+} and initially added 2–4 × 10^{-4} *M* **C** and 1 × 10^{-5} *M* PNPA. Hydrolysis of PNPA catalyzed by Zn(II)**C** or Zn(II)**D** was measured with initially added 1 × 10^{-5} *M* **C** or **D** and 1–5 × 10^{-4} *M* PNPA. The reactions were followed spectrophotometrically for the release of *p*-nitrophenol (PNP). Pseudo-first-order rate constants were calculated with the infinity absorbance readings measured.

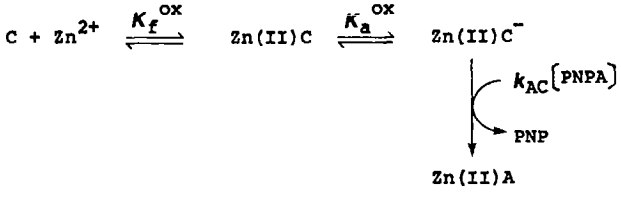
RESULTS

The kinetic data for the hydrolysis of **A** or **B**, the acyl intermediate which would be formed by the nucleophilic attack of **C** or **D** at PNPA, have been obtained in the presence of Zn^{2+} (*8, 10*). The pseudo-first-order rate constant for the Zn^{2+}-catalyzed deacylation (k_D) of **A** was represented by (*10*).

$$k_D = k_w[Zn^{2+}] + k_{OH}[Zn^{2+}][OH^-] + k_{te}[Zn^{2+}]^2[OH^-]^2 \qquad [1]$$

For **B**, only the k_{OH} term of Eq. [1] has been observed (*8*).

The formation of **A** from the reaction of **C** and PNPA was studied in the presence of Zn^{2+} at pH 6–7 by measuring the release of PNP under the condition of $[\mathbf{C}]_0 \gg [\text{PNPA}]_0$. The rate data were analyzed according to



[2]

TABLE 1

PARAMETER VALUES FOR ACETYLATION OF Zn(II)-COMPLEXED OXIMES WITH PNPA

	Zn(II)**C**[a]	Zn(II)**D**[b]
pK_a^{ox}	7.0 ± 0.1[c]	6.5 ± 0.1[c]
k_{AC} (sec^{-1} M^{-1})	400 ± 40	10 ± 2
K_f^{ox} (M^{-1})	420 ± 40	150 ± 15

[a] Measured at 25°C and an ionic strength of 1.0 in the presence of 0.8 %(v/v) acetonitrile and 0.02 *M* Mes.

[b] Ref. (*13*).

[c] pK_a for **C** and **D** (ionization of the oxime group) are 10.8 (measured by spectral titration in this study) and 10.0 (*13*), respectively.

and the estimated parameter values are summarized in Table 1.[2] The scheme of Eq. [2] assumes that the ionization of the oxime group is needed for the nucleophilic attack at PNPA. Also included in Table 1 are the parameter values measured (*13*) for the acetylation of **D** with PNPA in the presence of Zn^{2+}.

The hydrolysis of PNPA catalyzed by Zn(II)**C** was studied under the condition of $[PNPA]_0 \gg [C]_0$. The release of PNP at pH 6.0 (Fig. 1) indicates the initial burst and the subsequent linear increase in [PNP], demonstrating that both the formation and the turnover (decomposition) of the covalent intermediate occur at much faster rates compared with the spontaneous hydrolysis. When the pH was raised from 6.0 to 7.0, we were not able to observe the initial bursts (pre-steady-state kinetics). This is due to the increased overall rate and the enhanced ratio of k_D/k_A, as will be shown later in Table 2. The steady-state kinetic data obtained at pH 7.0 are illustrated in Fig. 2.

The initial burst and the steady-state kinetic data are analyzed according to

$$C + PNPA \xrightarrow{k_A} A \xrightarrow{k_D} C + AcO^- \qquad [3]$$
$$\downarrow PNP$$

As described earlier, the rate constants for the acylation (k_A) and the deacylation (k_D) steps are the functions of $[Zn^{2+}]$, pH, K_f^{ox} and K_a^{ox}. Under the condition of $S_0 \approx [S]$ (S; PNPA), the release of PNP follows

$$[PNP] = At + \pi(1 - e^{-bt}) \qquad [4]$$

$$A = k_D C_0 S_0/(k_D/k_A + S_0) \qquad [5]$$

[2] Over the pH range where ionization of **C** is negligible, the second-order rate constant for the acylation of **C** with PNPA (k_A of Eq. [3]) is related to the parameters of Eq. [2] as $k_A = k_{AC}/(1 + [H^+]/K_a^{ox} + [H^+]/K_a^{ox} \cdot K_f^{ox} \cdot [Zn^{2+}])$. A linear plot of $1/k_A$ measured at a fixed pH against $1/[Zn^{2+}]$ produces an intercept of $(1 + [H^+]/K_a^{ox})/k_{AC}$ and a slope of $[H^+]/K_a^{ox} \cdot K_f^{OH} \cdot k_{AC}$, from whose pH dependence the values of parameters listed in Table 1 for **C** were obtained.

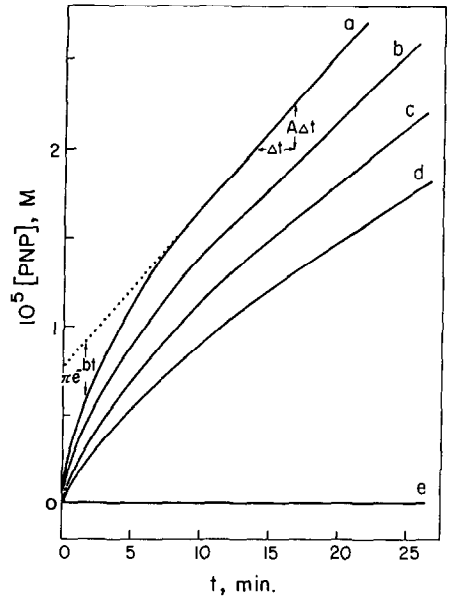


FIG. 1. Release of PNP from the hydrolysis of PNPA catalyzed by Zn(II)**C** at pH 6.0 with 0.01 *M* Zn^{2+} and 1.05×10^{-5} *M* **C**. Physical meanings of *A*, *b*, and π are also indicated. Data illustrated in this figure are those measured with S_0 of 3.04×10^{-4} *M* (a), 2.15×10^{-4} *M* (b), 1.46×10^{-4} *M* (c), and 1.00×10^{-4} *M* (d). Also included are the data for spontaneous hydrolysis (e; $S_0 = 1.00 \times 10^{-4}$ *M*).

TABLE 2

PARAMETER VALUES FOR PNPA HYDROLYSIS CATALYZED BY Zn(II)**C**[a,b]

	pH	
	6	7
$[Zn^{2+}]$ (*M*)	0.01	0.01
k_A (sec^{-1} M^{-1})	14.5 ± 0.5[c]	
	16.5 ± 0.5[d]	190 ± 30[e]
	(16.7)[f]	(180)[f]
10^3 k_D (sec^{-1})	2.11 ± 0.08[c]	
	1.34 ± 0.09[d]	35 ± 11[e]
	(1.61)[g]	(35)[g]
10^6 k_{sp} (sec^{-1})[h]	0.70	2.0

[a] Measured at 25°C and an ionic strength of 1.0 in the presence of 0.02 *M* Mes and 0.8 %(v/v) acetonitrile with $[PNPA]_0 \gg [Zn(II)C]_0$.

[b] For the Zn(II)**D**-catalyzed hydrolysis, $k_A = 5.0 \pm 0.1$ sec^{-1} M^{-1} and $k_D = (1.7 \pm 0.1) \times 10^{-4}$ sec^{-1} at pH 6.0 in the presence of 0.01 *M* Zn^{2+}.

[c] From the plot of C_0/A against $1/S_0$.

[d] From the plot of *b* against S_0.

[e] From the plot of C_0/k_0^i against S_0.

[f] Obtained from the reaction of PNPA with excessive amounts of Zn(II)**C**.

[g] Obtained from the Zn^{2+}-catalyzed hydrolysis of **A**.

[h] For spontaneous hydrolysis (W. P. Jencks and J. Carriuolo, *J. Amer. Chem. Soc.* (1960) **82**, 1778).

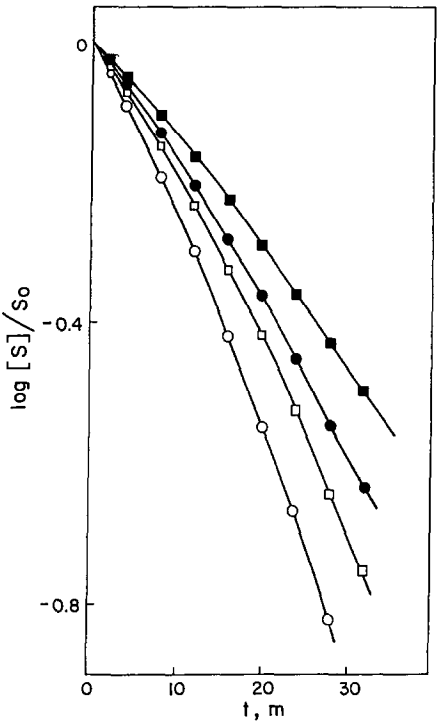


FIG. 2. Rate data for Zn(II)C-catalyzed hydrolysis of PNPA at pH 7 with 0.01 M Zn^{2+} and 9.77×10^{-6} M **C**. The decrease in log $[S]/S_0$ is plotted in order to emphasize that the reactions deviate from pseudo-first-order kinetics and that the initial pseudo-first-order rate constant (k_0^i) decreases as S_0 increases. Data illustrated are those measured with S_0 of 2.07×10^{-4} M (○), 3.30×10^{-4} M (□), 4.10×10^{-4} M (●), and 5.15×10^{-4} M (■).

$$b = k_D + k_A S_0 \quad [6]$$

$$\pi = C_0 S_0^2/(k_D/k_A + S_0)^2 \quad [7]$$

This equation has also been used for enzyme (*14*)- or polymer (*15*)-catalyzed reactions which proceed through the formation of covalent intermediates. Here, C_0 is the initially added concentration of the oxime. For the kinetic data of Fig. 1, the linear plots of b against S_0 (Fig. 3), C_0/A against $1/S_0$, and $\sqrt{C_0}/\sqrt{\pi}$ against $1/S_0$ (Fig. 4) give the values of k_A and k_D, which are summarized in Table 2.

The steady-state kinetic data of Fig. 2 are analyzed according to (*14*)

$$-d[S]/dt = k_D C_0[S]/(k_D/k_A + [S]) \quad [8]$$

Curvature (deviation from pseudo-first-order reactions) in the plots of Fig. 2 is as predicted by Eq. [8]. The linear plot of $1/k_0^i$ ($k_0^i = k_D C_0/(k_D/k_A + S_0)$; initial pseudo-first-order rate constant) against S_0 (Fig. 5) leads to the values of k_A and k_D, which are summarized in Table 2. The rate expression of Eq. [8] is identical with that of a simple Michaelis–Menten scheme, and k_D, k_D/k_A, and k_A correspond kinetically to enzymatic parameters k_{cat}, K_m, and k_{cat}/K_m, respectively.

If a part of PNPA is hydrolyzed without the formation of the *O*-acetyl oxime (e.g., nucleophilic attack by the metal-bound hydroxide of Zn(II)C), the relevant

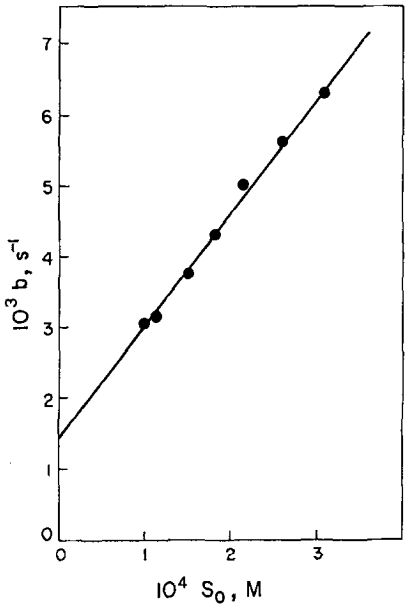


FIG. 3. The plot of b against S_0 for the data of Fig. 1.

rate expression indicates that k_A obtained from the plot of b against S_0 should be significantly smaller than the acylation rate constant ($k_A + k'_A$; k'_A stands for the path without the formation of **A**) measured under the condition of $C_0 \gg S_0$, and that the intercept of the plot of $\sqrt{C_0}/\sqrt{\pi}$ against $1/S_0$ should be considerably smaller than 1 ($1/\sqrt{1 + k'_A/k_A}$). However, the k_A obtained with the burst kinetic

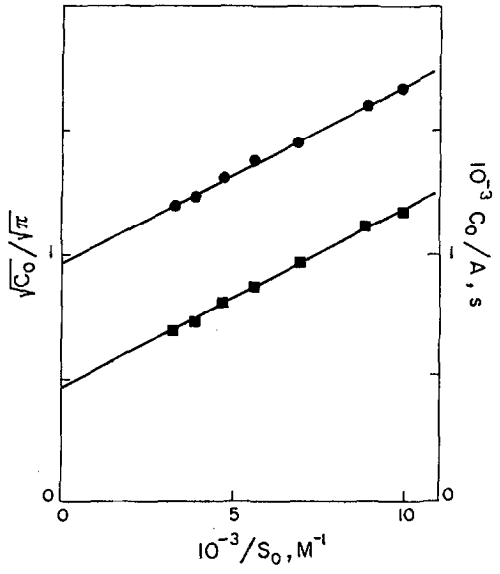


FIG. 4. The plots of C_0/A against $1/S_0$ (■) and $\sqrt{C_0}/\sqrt{\pi}$ against $1/S_0$ (●) for the data of Fig. 1.

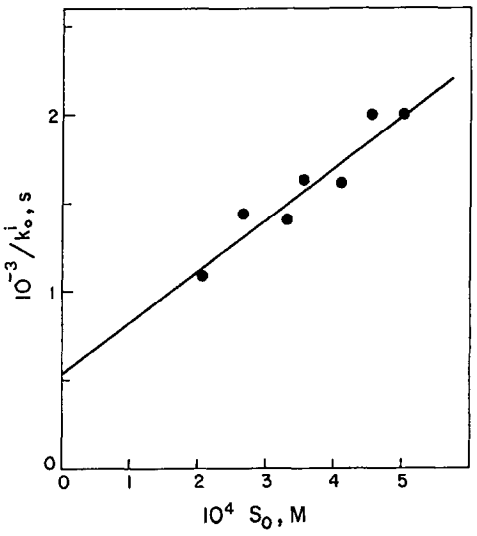


FIG. 5. The plot of $1/k_0^i$ against S_0 for the data of Fig. 2. C_0 is fixed at 9.77×10^{-6} M.

data agrees well with that obtained with excessive amounts of the catalyst, and the plot of $\sqrt{C_0}/\sqrt{\pi}$ against $1/S_0$ gives an intercept of 0.982 ± 0.015. This indicates that PNPA is hydrolyzed exclusively through the formation of **A**.

The hydrolysis of PNPA in the presence of Zn(II)**D** also follows Eq. [3]. Initial burst kinetics similar to those illustrated in Fig. 1 are observed at pH 6.0, although the rates are much slower compared with the Zn(II)**C**-catalyzed reaction. The values of k_A and k_D obtained by the analysis of the rate data measured under the condition of $S_0 \gg C_0$ agree well with the reported parameter values for acetylation (*13*) of Zn(II)**D** and the Zn^{2+}-catalyzed hydrolysis (*8*) of **B**. Typical values are listed in Table 2.

In the presence of 0.01 M Cu^{2+} at pH 4.0, the hydrolysis of PNPA is not catalyzed by **C** or **D** (1 mM). Under these conditions, the oximes are almost fully complexed with Cu^{2+}, and the oxime groups of the cupric complexes are mainly in the anionic form (*9, 16*).

DISCUSSION

A great deal of effort has been recently made to devise "artificial enzymes." Attempts to use "host–guest" systems to achieve binding and very close proximity between the nucleophilic functional group of the catalyst and the carbonyl carbon of the ester substrates often led to very fast acylation steps, but without turnover of the resulting acyl–catalyst intermediates. This is exemplified by the very fast acylation of crown ether-like compounds (*17*) or cyclodextrin derivatives (*18*). In designing nucleophilic catalysts, therefore, the ultimate goal is to make both the formation and the breakdown of the covalent intermediate to proceed much faster than the spontaneous reaction (*19*). This goal is achieved

with the present catalysts. Thus, the Zn(II)**C**- or Zn(II)**D**-catalyzed hydrolysis of PNPA at pH 6–7 proceeds exclusively through the formation of a covalent intermediate (**A** or **B**), and both the formation and the breakdown of the intermediate are much faster than the spontaneous hydrolysis of PNPA.

The much greater catalytic efficiency of Zn(II)**C** compared with Zn(II)**D** is the result of faster rates in both the acylation and the deacylation steps. The inability of Cu(II)**C** and Cu(II)**D** to catalyze the hydrolysis of PNPA in spite of the extremely fast breakdown of the respective covalent intermediates (*9, 10*) is due to the low basicity (*9*) of the Cu(II)-bound oximate ions and the conseqent very slow acylation rates. These again demonstrate the importance of acceleration in both acylation and deacylation steps in nucleophilic catalysis.

As mentioned earlier, k_A, k_D, and k_D/k_A correspond kinetically to enzymatic parameters k_{cat}/K_m, k_{cat}, and K_m, respectively. In this regard, the model catalysts employed in the present study are not as effective as proteolytic enzymes, considering that the enzymes hydrolyze their respective specific substrates with k_{cat}, K_m, and k_{cat}/K_m of about 10^2 sec^{-1}, 10^{-4} M, and 10^6 sec^{-1} M^{-1}, respectively. However, the rate parameters of the present model catalysts can be raised significantly with changes in $[Zn^{2+}]$ and pH.

In addition to the kinetic similarities, the present model exhibits the multifunctional nature of the catalysis, another characteristic of enzymes. As illustrated in Fig. 6, the Zn(II) ion in Zn(II)**C** acts as a Lewis acid to enhance the ionization of

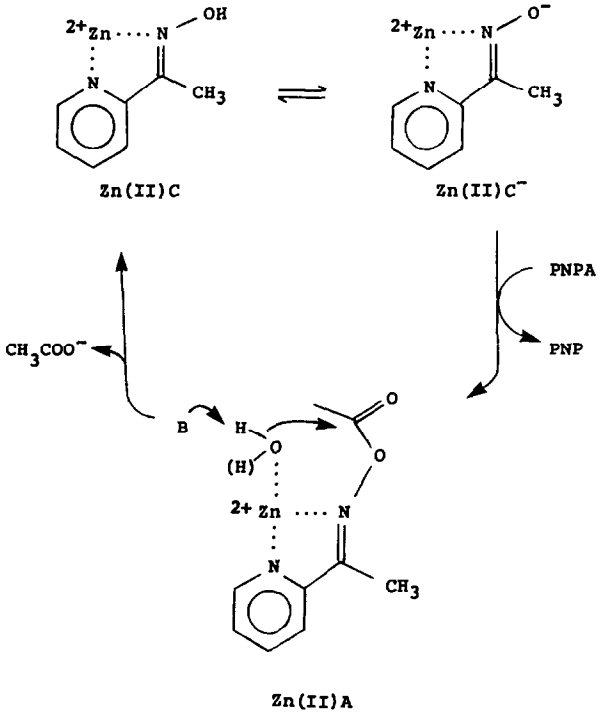


FIGURE 6

the oxime group (Table 1), and the oximate anion of Zn(II)$\mathbf{C}^-$ acts as the nucleophile in the acylation step. In the deacylation step, the Zn(II) ion in Zn(II)**A** acts as a Lewis acid to increase the leaving ability of the oximate group and as a template for the intramolecular attack of the nucleophiles at the complexed ester (*8, 9*). In addition, Zn(II)-bound water molecules and hydroxide ions of Zn(II)**A** are involved as nucleophiles, and the solvent molecule and free hydroxozinc(II) ion as general bases in the deacylation step (*9, 10*). The methyl group of **C** enhances the acylation rate by raising the nucleophilicity of the oximate anion through electronic effects and accelerates the deacylation step by exerting steric effects (*10*).

ACKNOWLEDGMENT

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