BIOORGANIC CHEMISTRY **12**, 177–187 (1984)

Zinc Ion-Catalyzed Ester Hydrolysis of *O*-Acetyl-2-acetylpyridineketoxime: Bimolecular Participation of Hydroxozinc(II) Ion

JUNGHUN SUH[1] AND HYUNSOO HAN

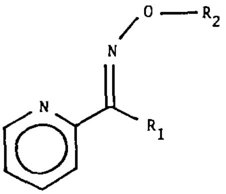
Department of Chemistry, Seoul National University, Seoul 151, Korea

Received May 16, 1983

The Zn^{2+}-catalyzed ester hydrolysis of *O*-acetyl-2-acetylpyridineketoxime (**A**) proceeds through three paths. The first two paths, the water path and the hydroxide path, have been observed in related systems. The third path, whose rate is bimolecular with respect to hydroxozinc(II) ion, is newly observed. The methyl group of **A** raises reaction rates through steric compression. The rate data obtained with **A** provide further evidences for the intramolecular nucleophilic attack of the metal-bound water molecule or hydroxide ion at the complexed ester. The third path is attributed either to the general base participation of free hydroxozinc(II) ion in the nucleophilic reaction of the Zn(II)-bound hydroxide ion or to the intramolecular reaction of dimeric hydroxozinc(II) ions. Implications of the present results to the actions of metalloenzymes, especially carbonic anhydrase and carboxypeptidase A, are also discussed.

Metal ion catalysis in the reactions of acyl derivatives has been studied extensively as an enzyme model, and various catalytic factors have been proposed (*1*). Our previous results on the Cu^{2+}-catalyzed hydrolysis of *O*-acetyl-2-pyridinecarboxaldoxime (**B**) provided evidences for a new catalytic factor, the nucleophilic attack by metal-bound water molecule (*2*).

In search of new catalytic factors in the metal ion-catalyzed transacylation reactions, we have made several structural variations with oxime esters. In this paper, the Zn^{2+}-catalyzed ester hydrolysis of *O*-acetyl-2-acetylpyridineketoxime (**A**) is reported. Kinetic data obtained in this reaction provide further evidence for the intramolecular nature of the reaction between the metal-bound nucleophile and the complexed ester. In addition, bimolecular participation of hydroxozinc(II) ion in the hydrolysis of the activated acyl derivative **A** is observed.



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.

0045-2068/84 $3.00

EXPERIMENTAL PROCEDURES

Materials. Compound **A** was prepared according to the literature (*3*), mp 64–65°C (Ref. (*3*), 65°C). 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 (*4*). 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. Buffers used were acetate (pH 4–4.5) and 4-morpholineethanesulfonate (Mes) (pH 5–7). The stock solution of **A** was made in acetonitrile and the reaction mixtures for kinetic studies contained 0.8% (v/v) acetonitrile. Metal ion-catalyzed hydrolysis of **A** was studied with initially added 1×10^{-4} *M* **A**. The reaction was followed spectrophotometrically for the release of M(II)**C**. Production of **C** in quantitative yields was evidenced by the product spectra (Zn(II)**C** or Cu(II)**C**) obtained at all of the pHs investigated. Pseudo-first-order rate constants were calculated with infinity absorbance readings measured.

RESULTS

The Zn^{2+}-catalyzed hydrolysis of **A** was kinetically studied at pH 4–7. The pseudo-first-order rate constants (k_o) were not affected appreciably by changes in buffer concentrations (2.5–10 m*M*). The pH dependence (Fig. 1) of k_o measured in the presence of 0.01 *M* Zn^{2+} indicated that k_o reached a limiting value ($k_W[Zn^{2+}]$) at

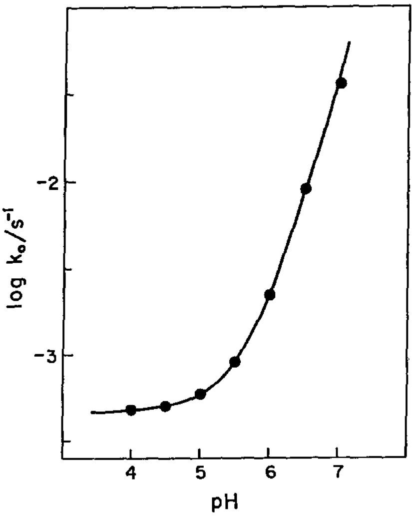


FIG. 1. pH dependence of k_o measured for the hydrolysis of **A** in the presence of 0.01 *M* Zn^{2+}.

low pH. For the rate data measured at a given pH, k_o was proportional to $[Zn^{2+}]$ at pH ≤ 5.5. This proportionality, however, did not hold at pH ≥ 6.0. Instead, $(k_o - k_W[Zn^{2+}])/[Zn^{2+}](= k')$ was linearly related to $[Zn^{2+}]$ (Fig. 2), with slope k'_{sl} and intercept k'_{int}

$$\begin{aligned} k' &= (k_o - k_W[Zn^{2+}])/[Zn^{2+}] \\ &= k'_{int} + k'_{sl}[Zn^{2+}] \\ &= k_{OH}[OH^-] + k_{te}[Zn^{2+}][OH^-]^2 \qquad [1] \end{aligned}$$

The plot of log k'_{int} against pH (pH 6.0–7.0) resulted in a straight line with slope 1, and that of log k'_{sl} against pH gave a straight line with slope 2 (Fig. 3). Thus, k'_{int} and k'_{sl} were proportional to $[OH^-]$ and $[OH^-]^2$, respectively. The rate data, therefore, are represented by Eq. [1], which is equivalent to

$$\begin{aligned} k_o &= k_W[Zn^{2+}] + k_{OH}[Zn^{2+}][OH^-] + k_{te}[Zn^{2+}]^2[OH^-]^2 \\ &= k_W[Zn^{2+}] + k_{OH}[Zn^{2+}][OH^-] + k_{bi}[Zn(II)OH^-]^2 \qquad [2] \end{aligned}$$

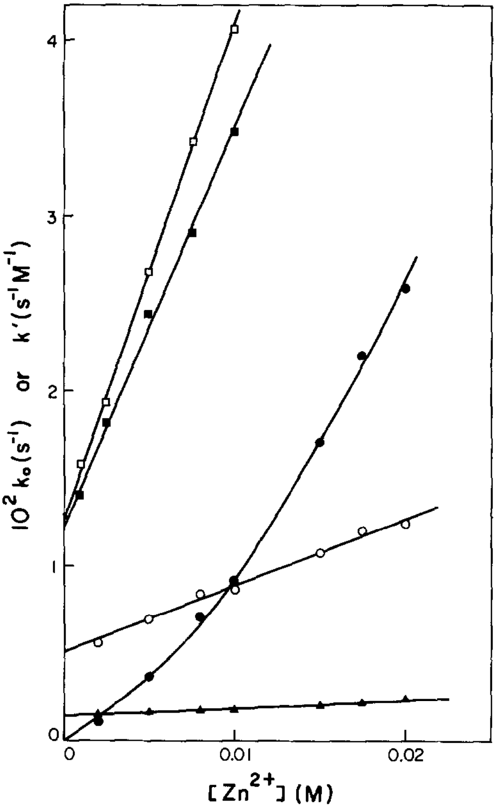


FIG. 2. Plot of k' (▲, pH 6.0; ○, pH 6.5; ■, pH 6.8; and □, pH 7.0) or k_0 (●, pH 6.5) for the Zn^{2+}-catalyzed hydrolysis of **A**.

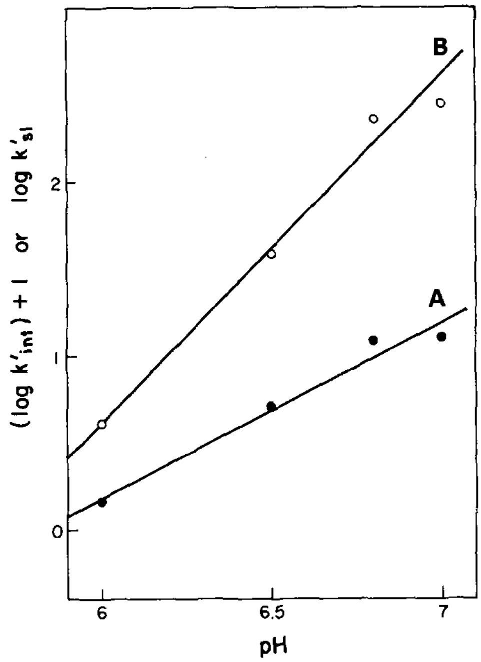


FIG. 3. Plot of log k'_{int} (A) or log k'_{sl} (B) against pH. (A) and (B) are drawn with slopes of 1.0 and 2.0, respectively.

The parameter values obtained from the analysis of the rate data according to Eqs. [1] and [2] are summarized in Table 1.

Previous studies on the metal ion-catalyzed hydrolysis of **B** established that the reaction involves the formation of the metal complex of the substrate (*2*, *5*). The K_f for Cu(II)**B** was large enough

$$\mathrm{M} + \mathrm{S} \overset{K_f}{\rightleftharpoons} \mathrm{MS} \xrightarrow{k_{\text{cat}}} \mathrm{M} + P_i \qquad [3]$$

to lead to saturation behavior of k_o with respect to [Cu^{2+}] (*2*). In the Zn^{2+}-catalyzed hydrolysis of **B**, however, k_o was proportional to [Zn^{2+}] because of the small K_f ($\leqslant 5\ M^{-1}$) and represented $K_f \cdot k_{\text{cat}}$ (*5*). Similarly, each parameter of Eq. [2] included K_f. Spectral titration (not shown) of **A** disclosed a pK_a of 2.9 ± 0.1 for the ionization of the pyridine ring. Over the pH range examined, therefore, the degree of complex formation was not affected appreciably by the ionization of **A**, and K_f could be taken as being constant. The K_f for Zn(II)**A** was also much smaller than 5 M^{-1}, as k_o measured at pH 4 was proportional to [Zn^{2+}] up to the highest concentration (0.2 *M*) attempted.

The parameter (k_W, k_{OH}, k_{te}/k_{bi}) values measured in the present study were much greater than those measured previously with **B** (*5*) (Table 1). This, however, does not necessarily reflect the relative reactivity (k_{cat}) of Zn(II)**A** and Zn(II)**B**, since each parameter contained K_f as well as k_{cat}. The k_{cat} values can be estimated with metal concentrations sufficiently larger than K_f^{-1} (*5*), although this is not possible with the Zn^{2+}-catalyzed reactions. On the other hand, k_{cat} for the Cu^{2+}-catalyzed

TABLE 1

PARAMETER VALUES FOR HYDROLYSIS OF OXIME ESTERS

	A[a,b]	**B**
Zn^{2+} catalyzed		
k_W (sec^{-1} M^{-1})	$(4.5 \pm 0.2) \times 10^{-2}$	—
k_{OH} (sec^{-1} M^{-2})	$(1.5 \pm 0.1) \times 10^7$	$(1.7 \pm 0.1) \times 10^6$[c]
k_{te} (sec^{-1} M^{-4})	$(4.1 \pm 0.3) \times 10^{16}$	—
k_{bi} (sec^{-1} M^{-2})	$(1.6 \pm 0.1) \times 10^6$[d]	—
Spontaneous[e]		
k_W (sec^{-1})	8.3×10^{-7}	15×10^{-7}
k_{OH} (sec^{-1} M^{-1})	8.2	15

[a] The Zn^{2+}-catalyzed reaction was measured at 25°C and an ionic strength of 1.0 in the presence of 0.8% (v/v) acetonitrile and 0.001–0.02 *M* Zn^{2+}.

[b] In the presence of 0.01 *M* Zn^{2+}, the hydrolysis of **A** was catalyzed by about 3×10^3 and 3×10^4 times at pH 6 and 7, respectively, compared with the spontaneous reaction.

[c] Ref. (*5*).

[d] Calculated by using pK_a of 8.8 (*6*) for Zn(II)OH_2.

[e] Calculated from the data reported in Ref. (*7*).

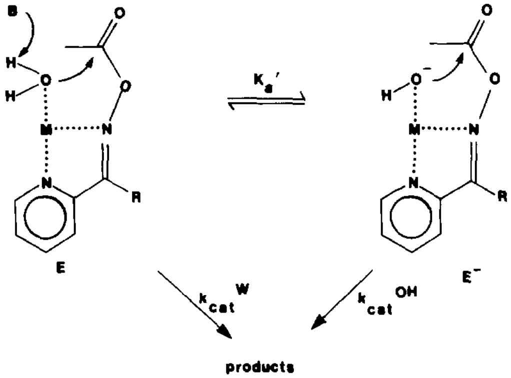
hydrolysis of **B** has been determined at relatively low [Cu^{2+}], and its pH dependence was similar to that illustrated in Fig. 1, except that the plateau was reached at pH ≤ 3 (*2*).

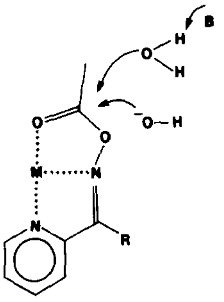
The hydrolysis of **A** occurred very rapidly in the presence of Cu^{2+}. Thus, the reaction was completed within the period of manual mixing (<5 sec) in the presence of 1 m*M* Cu^{2+} at pH 2.0, and detailed kinetic measurements were not performed. This observation, however, at least indicates that the k_{cat} for Cu(II)**A** is much greater than that for Cu(II)**B**. This is because the half-life of Cu(II)**B** is about 40 sec at pH 2–3 under the condition of complete complex formation ([Cu^{2+}] > 0.05 *M*) (*2*). Similarly, the greater parameter values for the Zn^{2+}-catalyzed hydrolysis of **A** compared with **B** may be also taken to indicate the larger k_{cat} values for Zn(II)**A**.

DISCUSSION

Metal Ion Catalysis in Oxime Ester Hydrolysis

The Zn^{2+}-catalyzed hydrolysis of **A** involves three reaction paths, each represented by k_W, k_{OH}, or k_{te}/k_{bi}. On the other hand, the k_{te}/k_{bi} path has not been observed in the Zn^{2+}- or Cu^{2+}-catalyzed hydrolysis of **B** (*2, 5*). Very slow reaction rates at low pHs have hampered the observation of the k_W path in the Zn^{2+}-catalyzed hydrolysis of **B**. Evidence has been presented (*2*) for the nucleophilic attack by the metal-bound water molecule or hydroxide ion (**E**/**E**$^-$), instead of the attack by external nucleophiles on the complexed carbonyl group (**F**), in the Cu^{2+}-catalyzed hydrolysis of **B**.





F

The methyl substituent in **C** raises the pK_a of the oxime group and its nucleophilicity toward nitrophenyl acetate (*8*). Similarly, the electronic effects of the methyl group in **A** lower the leaving group ability of the oximate ion, resulting in the slower rates of spontaneous hydrolysis compared with **B** (Table 1). On the contrary, M(II)**A** is hydrolyzed much faster (greater k_{cat}) than M(II)**B** when M is Cu or probably Zn, too.

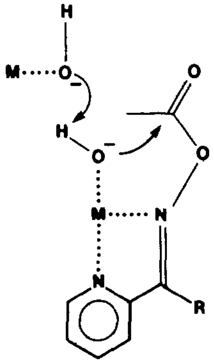
This can be explained in terms of steric compression between the two reaction centers in the intramolecular reaction of the metal-bound water molecule or hydroxide ion. The nonbonded interaction of the methyl group with the acyl moiety and with the adjacent hydrogen of the pyridine ring would push the carbonyl carbon toward the metal-bound nucleophile oxygen atom. The steric compression by the methyl substituent is supported by the X-ray crystal structures of the Pt(II) complexes of **C** and **D** (*9, 10*). Similar acceleration by steric compression has also been observed in other model systems. For example, about a 10^{10}-fold rate change in the amide hydrolysis of maleamic acid derivatives was achieved when the alkyl substituents on the vinylic carbons were varied (*11, 12*). The steric effect of the methyl substituent is more consistent with the mechanism of **E**/**E**$^-$ than that of **F**. This is because a five-membered ring which is absent in the ground state is formed in the transition state of the mechanism of **E**/**E**$^-$, while loosening of the five-membered ring is involved in that of **F**. The greater k_{cat} values for both the k_W and the k_{OH} paths for M(II)**A** compared with M(II)**B**, therefore, provide further support for the validity of mechanism **E**/**E**$^-$.

When the pK'_a of Zn(II)**A** in **E** is approximated as the pK_a (8.8 (*6*)) of Zn(II)OH$_2$, the rate constants listed in Table 1 [$k_W = k_{cat}^W \cdot K_f$; $k_{OH} = k_{cat}^{OH} \cdot K'_a \cdot (K_f/K_W)$] indicates that the reactivity of the Zn(II)-bound hydroxide ion (k_{cat}^{OH}) is only about 2000 times greater than that (k_{cat}^W) of the Zn(II)-bound water molecule. The general base assistance by a solvent molecule (*2*) to the attack of the metal-bound water could be very effective since the nucleophilic water molecule is bound to a metal ion. Thus, the low selectivity manifested by the metal-bound water molecule and hydroxide ion is not surprising. In this regard, it is noteworthy that the β_{Nu} values obtained by the plot of log k against the pK_a of the metal-bound water molecule in the nucleophilic attack of metal-bound hydroxide ions at propionic anhydride and carbon dioxide are only 0.25 and 0.18, respectively (*13*).

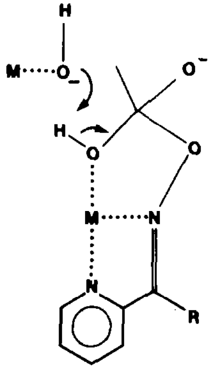
It is difficult to rationalize the k_{te}/k_{bi} path in terms of the mechanism of **F**. If the reaction occurs through this mechanism and the attack of the external hydroxide ion is rate controlling, the improbable assistance by Zn(II)OH$^-$ or by Zn^{2+} and OH$^-$ to the attack of the free hydroxide ion is required to explain the k_{te}/k_{bi} path. Even when a discrete tetrahedral intermediate exists in the mechanism of **F**, the rate-controlling step must be the formation of the tetrahedral intermediate considering the relative leaving ability of the hydroxide ion and the oximate anion of Zn(II)**C** (pK_a = 7.0 (*8*)). The breakdown of the intermediate might be catalyzed by Zn(II)OH$^-$, but this cannot increase the overall rate.

With the mechanism of **E**$^-$ operating for the k_{OH} path, a probable mechanism for the k_{te}/k_{bi} path can be presented by either **G** or **H**. In **H**, the proton abstraction and the expulsion of the leaving group may be either concerted or stepwise. The relative leaving ability of the Zn(II)-bound oximate anion and the Zn(II)-bound hydroxide ion from the tetrahedral intermediate formed in the reaction of **E**$^-$ is not easily predicted, as the difference in the pK_as of the leaving groups is not very large (*14*, *15*). However, Zn(II)OH$^-$ can participate as a general base either in the formation (**G**) or in the breakdown (**H**) of the tetrahedral intermediate. The mechanisms of **G** and **H** are similar to those proposed for the bimolecular participation of amines in the aminolysis of esters or for the bimolecular participation of hydroxide ion in the alkaline hydrolysis of anilides (*16–21*). Furthermore, the possibility has been mentioned that metal-bound hydroxide ion might be further deprotonated after it attacks the carbonyl group in the Zn^{2+}-catalyzed hydrolysis of anhydrides (*22*).

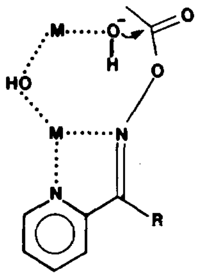
Both the binding (K_f) of the substrate to the metal ion and the catalytic conversion (k_{cat}) of the resultant metal–substrate complex can be affected by the added buffer bases. Metal binding of the added bases would lower the overall rate by blocking the binding sites on the metal ion for the substrate. On the other hand, general base assistance by the bases in the k_{cat} step would raise the overall rate. How these two conflicting effects would compromise is not easy to predict, as discussed previously in the Cu^{2+}-catalyzed hydrolysis of **B** (*2*). In the present study, therefore, kinetic data are obtained under the condition that a change in buffer concentration does not affect the overall rate. Over the pH range 6–7 where the k_{te}/k_{bi} path is observed, Mes, a tertiary amine, is used as the buffer. Mes



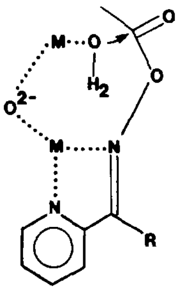
G



H



I



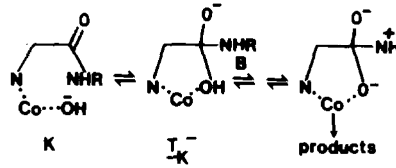
J

concentration does not affect the overall rate, apparently because of the bulky substituents on its nitrogen atom. The steric inhibition imposed by the *N*-substituents should prevent both metal coordination and general base participation by Mes. It was not attempted to observe catalysis depicted in **G** or **H** by bases other than the hydroxozinc(II) ion in the present study.

As long as catalysis by other bases is not observed, the mechanism of **G**/**H** is not firmly established. In this regard, the participation of dimeric hydroxozinc(II) ions in the k_{te}/k_{bi} path is not precluded. Two possible mechanisms, which lead to the bimolecular kinetic expression with respect to hydroxozinc(II) ion, are illustrated by **I** and **J**. In these mechanisms, binding of the substrate to one of the Zn atoms precedes the intramolecular attack by the Zn(II)-bound nucleophiles. Direct attack of the dimeric hydroxozinc(II) ions at the uncoordinated substrate can be excluded on the grounds that the k_{te}/k_{bi} term is not observed for **B** (*5*), which is intrinsically more labile than **A**. The equilibrium constants for dimerization of the hydroxozinc(II) ion and the consequent equilibrium concentrations of the dimeric species are needed in analyzing the rate data in terms of mechanism **I** or **J**. These quantities, however, are not available at present.

Implications to Metalloenzymes

Previously, we presented evidence for the general base catalysis in the intramolecular attack of metal-bound water molecule at an acyl derivative (*2*). The present results suggest that the general base catalysis could be extended to the intramolecular attack of metal-bound hydroxide ion. Geneal base catalysis in the reaction of metal-bound hydroxide ion has also been reported for the hydrolysis of Co(III)-complexed amides (*23*). In this reaction, the bases participate in proton transfer between tetrahedral intermediates in the process of protonation of the leaving nitrogen atom.[2]



[4]

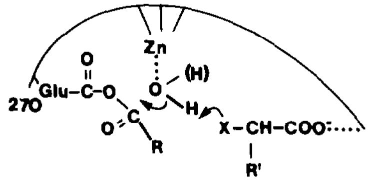
The present system stands in contrast to the Co(III) system as it involves the hydrolysis of activated acyl derivatives. In enzymatic reactions, a series of labile acyl intermediates are likely involved in the transformation of stable esters or amides into the hydrolysis products. Since every step in an enzymatic reaction must be very fast, the reaction of even the metal-bound hydroxide ion with a labile acyl-enzyme intermediate could require general base assistance.

General base catalysis in the attack of the water molecule or hydroxide ion bound to the active-site Zn(II) ion at carbon dioxide is usually assumed in the proposed mechanisms of carbonic anhydrase (*25*). Experimental demonstration of this catalytic factor with small molecules is, therefore, of great importance. When analyzed in terms of the mechanisms of **G** or **H**, the k_{bi} value (1.6×10^6 sec^{-1} M^{-1}) corresponds to $K_f' \cdot k_{cat}'$ (K_f' for complex formation between **A** and Zn(II)OH$^-$; k_{cat}' for the reaction of Zn(II)OH$^-$ · **A** in the presence of Zn(II)OH$^-$). From this, although the value of K_f' is unknown ($K_f \leqslant 5\ M^{-1}$ for Zn(II)**A**), K_{cat}' can be compared with the reactivity ($>10^5$ sec^{-1}) of carbonic anhydrase toward bound carbon dioxide.

Previously, we proposed general base catalysis in the attack of the Zn(II)-bound water molecule at acyl-carboxypeptidase A intermediates (*2*). The present results suggest that the proposal of the general base catalysis could be extended to the attack of the Zn(II)-bound hydroxide ion at the acyl-carboxypeptidase A interme-

---

[2] In Ref. (*23*) the cyclization step of Eq. [4] was assumed to be rate controlling. According to the analysis made in the same paper, however, k_{10}[**B**] and k_{-3} (k's in this footnote are those listed in Scheme V of Ref. (*23*)) are estimated as about 3×10^4 sec^{-1}. Since k_{-2} must be much greater than k_{-3}, $k_{-2} \gg k_{10}$[**B**] and the cyclization step of Eq. [4] is in preequilibrium. The product distribution reported in Ref. (*23*) is also explained even if the k_{10} step instead of the k_2 step is considered to be the rate-controlling step. It was proposed that a Co(III)-bound water molecule makes an intramolecular nucleophilic attack at the amide group at low pHs (*23*). The results obtained at low pHs with the Co(III)-catalyzed amide hydrolysis as well as analogous lactonization (*24*), however, are equally well or better explained by the kinetically equivalent intramolecular nucleophilic attack of the Co(III)-bound hydroxide ion combined with the participation of the specific acid.



L (X = NH_2 or OH)

diates as illustrated in **L**. This is analogous to the general base catalysis proposed for carbonic anhydrase.

One of the major controversies about the mechanism of carboxypeptidase A is the role of Glu_{270} carboxylate. Although spectroscopic detection of an acyl-enzyme intermediate has been reported in the hydrolysis of an ester substrate (*26*), the strongest evidence in support of the general base role of the Glu_{270} carboxylate, at least in peptide hydrolysis, came from the ^{18}O-exchange experiments of Breslow and Wernick (*27*). The ^{18}O-exchange results, however, can be explained in terms of the nucleophilic role of Glu_{270} if "acyl-enzyme hydrolysis and resynthesis are strongly catalyzed by enzyme-bound amino acids but not by enzyme-bound hydroxy acids" (footnote 7 of Ref. (*27*)). According to **L**, the hydrolysis and, consequently, the resynthesis of the acyl-carboxypeptidase A intermediate, could be catalyzed much more strongly by the enzyme-bound amino acids because of their much greater basicity. The ^{18}O-exchange results, therefore, do not necessarily support the general base role of the Glu_{270} carboxylate.

ACKNOWLEDGMENT

This work was supported by a grant from the Korea Science and Engineering Foundation.

REFERENCES

1. Satchell, D. P. N., and Satchell, R. S. (1979) *Ann. Rep. Progr. Chem. Sect. A* **75,** 25–48, and references therein.
2. Suh, J., Cheong, M., and Suh, M. P. (1982) *J. Amer. Chem. Soc.* **104,** 1654.
3. Blanch, J. H., and Onsager, O. T. (1965) *J. Chem. Soc.* 3734.
4. Perrin, D. D., Armarego, W. L. F., and Perrin, D. R. (1966) Purification of Laboratory Chemical, 1st ed., p. 58, Pergamon, London.
5. Suh, J., Lee, E., Jang, E. S. (1981) *Inorg. Chem.* **20,** 1932.
6. Basolo, F., and Pearson, R. G. (1962) Mechanisms of Inorganic Reactions, 2nd ed, p. 32, Wiley, New York.
7. Blanch, J. H. (1968) *J. Chem. Soc.* (*B*) 167.
8. Suh, J., Cheong, M., and Han, H. (1984) *Bioorg. Chem.* **12,** 188–196.
9. Nordquest, K. W., Phelps, D. W., Little, W. F., and Hodgson, D. J. (1976) *J. Amer. Chem. Soc.* **98,** 1104.
10. Phelps, D. W., Little, W. F., and Hodgson, D. J. (1976) *Inorg. Chem.* **15,** 2263.

11. Kirby, A. J., and Fersht, A. R. (1971) *Prog. Bioorg. Chem.* **1,** 1.

12. Kirby, A. J., and Lancaster, P. W. (1972) *J. Chem. Soc. Perkin Trans. 2* 1206.

13. Martin, R. B. J. (1976) *J. Inorg. Nuc. Chem.* **38,** 511.

14. Gresser, M. J., and Jencks, W. P. (1977) *J. Amer. Chem. Soc.* **99,** 6970.

15. Suh, J., and Lee, B. H. (1980) *J. Org. Chem.* **45,** 3103.

16. Bruice, P. Y., and Bruice, T. C. (1974) *J. Amer. Chem. Soc.* **96,** 5533.

17. Satterthwait, A. C., and Jencks, W. P. (1974) *J. Amer. Chem. Soc.* **96,** 7018.

18. Jencks, W. P., Oakenfull, D. G., and Salvesen, K. J. (1970) *J. Amer. Chem. Soc.* **92,** 3201.

19. Kershner, L. O., and Schowen, R. L. (1971). *J. Amer. Chem. Soc.* **93,** 2014.

20. Pollack, R. M., and Bender, M. L. (1970) *J. Amer. Chem. Soc.* **92,** 7191.

21. Menger, F. M., and Donahue, J. A. (1973) *J. Amer. Chem. Soc.* **95,** 432.

22. Breslow, R., McClure, D. E., Brown, R. S., and Eisenach, J. (1975) *J. Amer. Chem. Soc.* **97,** 194.

23. Boreham, C. J., Buckingham, D. A., and Keene, F. R. (1979) *J. Amer. Chem. Soc.* **101,** 1409.

24. Boreham, C. J., Buckingham, D. A. Francis, D. J., Sargeson, A. M., and Warner, L. G. (1981) *J. Amer. Chem. Soc.* **103,** 1975.

25. Pocker, Y., and Sarkanan, S. (1978) *Advan. Enzymol.* **47,** 149.

26. Makinen, M. W., Yamamura, K., and Kaiser, E. T. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 3882.

27. Breslow, R., and Wernick, D. (1976) *J. Amer. Chem. Soc.* **98,** 259.