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REVIEW

Model Studies of Carboxypeptidase A[1]

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In the action of carboxypeptidase A (CPA), the active-site Zn(II) ion and the Glu-270 carboxylate group are believed to play very crucial catalytic roles. As models of CPA, various aspects of catalysis of amide or ester hydrolysis by the carboxyl group or the metal ion as the monofunctional catalytic group have been intensively investigated. On the other hand, the cooperative catalysis by the carboxyl group and the metal ion has been achieved in few model reactions. In some of the bifunctional catalytic systems, however, the catalytic roles of the carboxyl group and the metal ion are different from those proposed for the action of CPA. The most successful model of CPA designed to date is the Ni(II)-catalyzed hydrolysis of ester **37** and the Cu(II)-catalyzed hydrolysis of amide **39** in dimethyl sulfoxide containing 5% (v/v) water. In these reactions, the following catalytic features of CPA are reproduced: (i) both an alkyl amide and an alkyl ester are readily hydrolyzed, (ii) the catalysis is achieved by the cooperative participation of the metal ion and the carboxyl group as well as the reaction medium, (iii) the catalytic roles of the metal ion and the carboxyl group are similar to those proposed for CPA action, and (iv) the anionic form, instead of the acidic form, of the carboxyl group is catalytic. Various model studies also provide mechanistic information with which the data obtained directly with CPA can be reevaluated. Some of the mechanistic arguments previously presented in support of the general base role of the Glu-270 carboxylate, when reevaluated in the light of results from the model studies, appear to be also compatible with the nucleophilic role of Glu-270.

INTRODUCTION

The main purpose of model studies of an enzyme is to reproduce the major characteristics of the enzyme, such as the ability to recognize substrate structures and to accelerate conversion of the substrates into the products. Recognition of the substrate structures by catalysts and formation of tight and productive catalyst–substrate complexes have been attempted (*1*, *2*). These model systems are designed to mimic the binding features of enzymes in general instead of a specific target enzyme. At present, model studies of a specific enzyme are usually focused on achieving highly efficient rate enhancement by using the catalytic principles employed by the target enzyme.

The model studies are also undertaken to resolve mechanistic ambiguities concerning the target enzyme. Since an enzymatic system is too complicated to

[1] Dedicated to the memory of Professor E. T. Kaiser.

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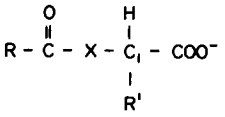
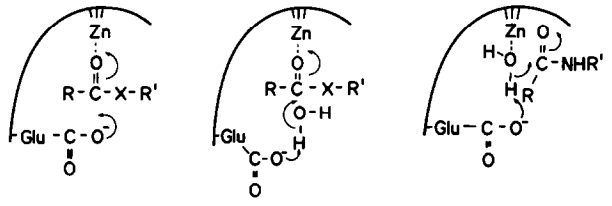
investigate directly, a few important features of the enzyme are incorporated into simple model systems and their mechanistic significance is tested. Sometimes, information obtained directly with the target enzyme is reevaluated with model compounds.

Carboxypeptidase A (CPA) (*3–5*) is a Zn(II)-metalloenzyme which catalyzes the hydrolysis of amide bonds adjacent to the C-terminal amino acid residue of the substrate (**1**). Ester or thiol ester analogues of the amide substrates are also hydrolyzed by CPA.



1 (Configuration of C_1 is L when X = NH or O.)

The mechanism of CPA action has been intensively investigated by using X-ray crystallographic (*5–10*), kinetic (*11–17*), spectroscopic (*18–22*), and mutagenetic (*23*) methods in addition to other (*24–28*) chemical methods. The active-site Zn(II) ion and the Glu-270 carboxylate of CPA are believed to be the essential catalytic groups in the hydrolysis of both ester and amide substrates. The Zn(II) ion is bound to two imidazole nitrogens of His-69 and His-196 as well as the carboxylate oxygen of Glu-72. Maximum activity of CPA is manifested at pH 7–8, and the anionic form, instead of the acidic form, of the Glu-270 carboxyl group is the catalytic species. The most widely proposed role of the Zn(II) ion is the polarization of the carbonyl group of the scissile ester or amide linkage by binding of the carbonyl oxygen. In a recently proposed mechanism for the proteolytic action of CPA, the water molecule bound to the Zn(II) ion makes a nucleophilic attack at the acyl carbon of the bound substrate (*5*). The carboxylate anion of Glu-270 is proposed to act as either a nucleophile or a general base, and the exact catalytic role has been the most controversial issue in the mechanistic study of CPA. The mechanisms most frequently proposed for CPA action are illustrated in **2–4**.



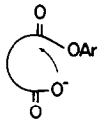
2 **3** **4**

Early model studies of CPA were focused on the examination of catalytic efficiency of the individual catalytic groups of CPA, and recent ones on the collabo-

ration among multiple catalytic factors. In this article, results of model studies concerning the intramolecular catalysis of ester and amide hydrolysis by the carboxyl group or the metal ion as the monofunctional catalyst are summarized, and those concerning the cooperative catalysis by the metal ion and the carboxyl group are presented. Some models also provide clues for interpretation of mechanistic data obtained directly with CPA and are also described in this article.

CATALYSIS BY THE CARBOXYL GROUP

Intramolecular nucleophilic catalysis by the carboxyl group has been extensively investigated with various types of reactions (*29–31*) including hydrolysis of esters and amides. For example, hydrolysis of aryl esters of various dicarboxylic acids has been studied (*30*), revealing that the rate for the nucleophilic attack (**5**) of the carboxylate anion increases remarkably as the conformational freedom of the dicarboxylic acid is reduced. Alkoxide anions are much more basic than phenolate anions, and, thus, expulsion of alkoxide anions from tetrahedral intermediates is much more difficult than that of phenolate anions. In the hydrolysis of monoalkyl esters of phthalate or maleate derivatives (*32–34*), protonation (e.g., **6**) of the leaving alkoxy oxygen facilitates the expulsion of alkoxide anions. Consequently, the overall kinetic behavior manifests apparent catalysis by the acidic form of the intramolecular carboxyl group, although the carboxylate anion makes nucleophilic attack at the acyl center. When the leaving alkoxide anion is weakly basic, protonation of the leaving group is not necessary.



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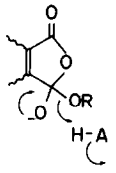

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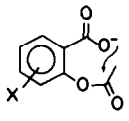
Mechanisms of intramolecular catalysis by carboxyl groups in the ester hydrolysis of aspirin derivatives have been intensively investigated (*35, 36*). In the hydrolysis of the monoesters of dicarboxylic acids (**5**), the leaving groups are detached from the reaction center once they are expelled from the tetrahedral intermediates. If the carboxylate group of an aspirin derivative makes nucleophilic attack at the ester linkage (**7**), however, the leaving phenolate group remains in the vicinity of the reaction center even after expulsion from the acyl carbon, and, consequently, the reverse attack (**8**) by the expelled phenolate anion decreases the overall rate. For the unsubstituted aspirin, the reverse attack is so effective that the ester bond is hydrolyzed through the general base catalysis (**9**), instead of the nucleophilic catalysis, by the intramolecular carboxylate ion.



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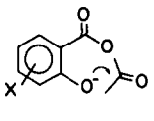

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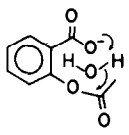

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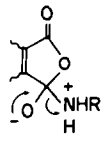
By using monoamides of maleic acid and related dicarboxylic acids, mechanistic information for the intramolecular catalysis by the carboxyl group in amide hydrolysis has been obtained (*37–43*). Hydrolysis of the amides is catalyzed only by the acidic form of the intramolecular carboxyl group, since the protonation of the leaving nitrogen atom is required for the expulsion of the amine from the tetrahedral intermediate (e.g., **10, 11**). Rates of the deacylation of monoalkyl amides of maleic acid derivatives vary remarkably when the substituents on the olefinic carbons of maleic acid are changed. The rate constant observed when the catalytic carboxyl group is fully in the acidic form is 1.1 s^{-1} at 39°C for amide **12**, the most reactive monoalkyl amide of maleic acid derivatives designed so far. On the other hand, the rate constant for amide **13** is 2.2 × 10^{-6} s^{-1} at 100°C. This rate difference has been attributed to the steric compression between the carboxyl oxygen and the amide carbon atoms and the consequent strain in the ground state, demonstrating the importance of the proximity effect in enzymatic catalysis.



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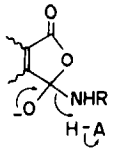

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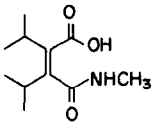

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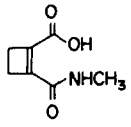

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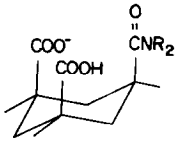
Continuous efforts have been made to design catalytic systems in which alkyl amide bonds are cleaved very effectively by the nucleophilic attack of intramolecular carboxyl groups. Recently, fast cleavage (half-life: 8 min at pD 7.05 and 21.5°C) of amide **14** at neutral pH has been reported (*44*). This amide is deacylated by the carboxyl group in the acidic form. Due to the presence of the adjacent carboxylate anion, the pK_a of the carboxyl group is raised to 6.9, and, thus, about half of the carboxyl group is in the acidic form at pH 7. Consequently, the amide is rapidly hydrolyzed at neutral pH's. The limiting rate constant measured for amide **14** with

the catalytic carboxyl group present entirely in the acidic form is 3.5×10^{-3} s^{-1} at 21.5°C, which is considerably smaller than that measured for amide **12**.



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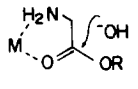
CATALYSIS BY THE METAL ION

The catalytic roles of the active-site Zn(II) ion of CPA indicated by **2–4** were proposed on the basis of X-ray crystallographic studies performed on unproductive and static complexes of CPA formed with a pseudo-substrate or ketonic inhibitors (*5–7*). At present, however, no physical tools are available for the elucidation of the exact catalytic role of the Zn(II) ion directly using the enzyme. In this regard, studies on the catalytic features of metal ions in transacylation reactions can provide valuable information for the catalytic role of the Zn(II) ion of CPA.

Metal ions catalyze hydrolysis of amides or esters by acting as Lewis acids (*45*). Hydronium ion also acts as a Lewis acid catalyst in the hydrolysis of amides and esters. Unlike hydronium ions, metal ions can be present in high concentrations even at neutral or alkaline pH's. In addition, metal ions can possess multiple positive charges, exerting greater electrostatic effects compared with protons. Furthermore, it is possible to make metal ions interact with a specific site on the substrate by properly designing the substrate structure. Another unique catalytic feature of metal ions unmatchable by protons is the template effect, which converts the intermolecular reactions between reacting sites into intramolecular processes.



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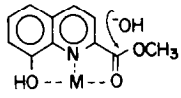

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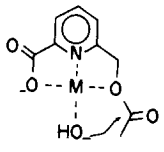

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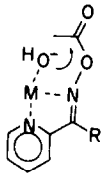
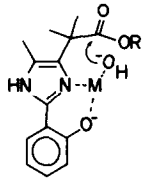


18

In the metal-ion-catalyzed hydrolysis, the catalytic species may be the metal ion itself, metal-bound hydroxide ion, or metal-bound water molecule. When the metal ion itself acts as a catalytic group, it may enhance the electrophilicity of the carbonyl group by binding the carbonyl oxygen as illustrated by **15** (*46*) and **16** (*47*). In some reactions, the metal ion raises the leaving ability of the leaving group by lowering the basicity of the leaving group as illustrated by **17** (*48*) and **18** (*49–52*).

When metal-bound hydroxide ions are the catalytic groups, they usually act as nucleophiles as illustrated by **19** (*53*) as well as **17** and **18**. Ionization of water is facilitated by coordination to metal ions (*54*), and considerable concentrations of metal-bound hydroxide ions can be obtained at neutral or acidic pH's.



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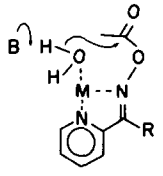

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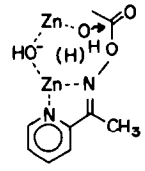
Although the nucleophilicity of water would decrease remarkably upon coordination to metal ions, nucleophilic attack **(20)** by a metal-bound water molecule at an ester linkage has been reported (*49*). Here, nucleophilic attack by the metal-bound water molecule is attributable to the template effects exerted by the metal ion and assistance from general bases.

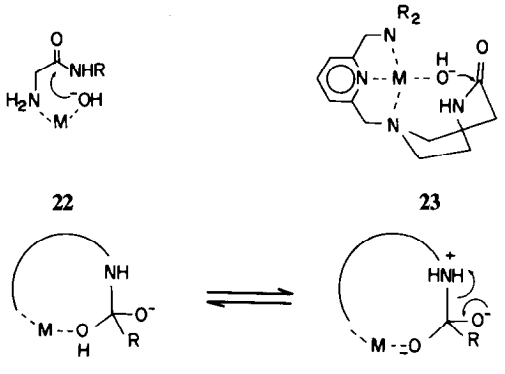
Binuclear metal ions also act as catalytic units, as illustrated by **21** (*55*). Catalysis by the binuclear metal ions in spite of their very low concentrations in water suggests that the geometry of the transition state involving the binuclear metal ion is well suited for the facile intramolecular process.



21

Amide linkages are much more difficult to hydrolyze than ester linkages. Metal ion catalysis in the hydrolysis of alkyl amides has been also achieved (**22** and **23**) (*56–58*). In **22** and **23**, the metal-bound hydroxide acts as the intramolecular

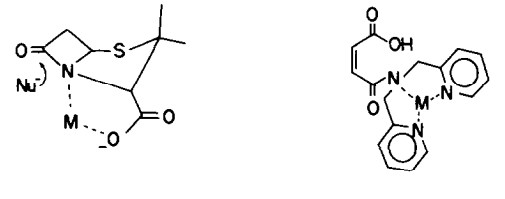
nucleophile and as the proton source needed in the expulsion (**24**) of the amines from the tetrahedral intermediates.



22 **23**

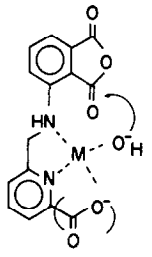
24

In amide hydrolysis, coordination of the leaving amine to a metal ion might lead to catalysis. This possibility has been tested with penicillin derivatives (*59, 60*) such as **25** as well as maleate monoalkyl amide **26** (*42*). Due to the ring strain of the β-lactam ring, the nitrogen atom on the lactam ring possesses the character of an amine nitrogen instead of an amide nitrogen. Consequently, the nitrogen atom of the penicillin derivative readily coordinates to metal ions, leading to catalysis in the amide cleavage. Maleic monoamide **26**, however, is hydrolyzed by the catalytic action of the carboxyl group, without assistance from added metal ions. Apparently, the binding of metal ions to the leaving nitrogen of **26** either in the amide substrate or in the tetrahedral intermediate is not efficient enough to compete with the reaction path (**10, 11**) involving hydronium ion.



25 **26**

If the Glu-270 carboxylate of CPA acts as a nucleophile, an anhydride intermediate is formed between Glu-270 and the acyl portion of the substrate. The breakdown process of the anhydride intermediate must be faster than the overall reaction. In this regard, the metal-ion catalysis of carboxyl anhydrides (**27, 28**) has been also investigated as CPA models (*61, 62*).



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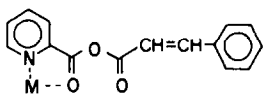

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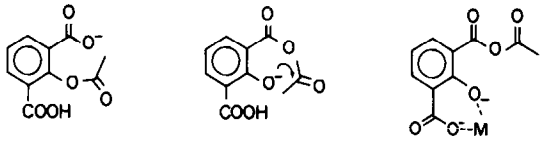
In the reactions exemplified above, metal ions, metal-bound hydroxide ions, or metal-bound water molecules perform catalytic roles to enhance the reactivity of acyl derivatives or nucleophiles. Catalysis by metal ions that simply block inhibitory reverse paths has been reported (*63*). In the hydrolysis of 3-carboxyaspirin (**29**), the reverse attack (**30**) by the phenolate anion at the anhydride linkage of the intermediate formed in the nucleophilic mechanism is so effective that the spontaneous hydrolysis of **29** involves the general base participation by the carboxyl group, in analogy with aspirin hydrolysis (**9**). Fe(III) or Al(III) ion accelerates the hydrolysis of **29** by blocking the inhibitory reverse attack of the phenolate anion at the anhydride linkage (**31**). Consequently, **29** is hydrolyzed through the nucleophilic participation, instead of general base assistance, by the intramolecular carboxylate group in the presence of the metal ions. The majority of enzymatic reactions involve covalent intermediates (*64*). When the enzymatic process is a substitution reaction, the leaving group of the substrate remains in the vicinity of the reaction site after it is cleaved by the attack of the enzymatic group. The reverse attack of the leaving group at the resultant intermediate, however, should be also very efficient if the leaving group remains in close proximity to the reaction site. Since this retards the overall reaction, the enzyme must separate the leaving group from the reaction site or block its reactivity. The model system of **31** suggests that the reverse attack by the leaving group could be blocked by using the active-site metal ion in the action of metalloenzymes.



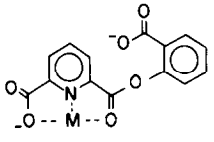
29 30 31

Binding of metal ions to proteins can result in changes in conformation and many other properties of the protein. In the case of CPA, substitution of the active-site Zn(II) ion with other metal ions leads to marked changes in kinetic behavior (*28, 65–68*). It is very difficult to explain the effects of metal substitution on the catalytic behavior of metalloenzymes. As a model for this aspect of metalloenzymes, it has

been reported that slight changes in the geometry around the central metal atom affect the catalytic outcome remarkably in metal ion-catalyzed hydrolysis of oxime esters related to **18** (*69*).

COOPERATIVE CATALYSIS BY CARBOXYL GROUP AND METAL ION

Intramolecular catalysis of ester or amide hydrolysis either by the carboxyl group or by the metal ion as the monofunctional catalyst has been achieved in a large number of model studies. On the other hand, attempts to achieve cooperative catalysis by the metal ion and the carboxyl or other (*70*) organic functional groups have been successful only in a few model studies. The metal ion catalysis of the hydrolysis of aspirin derivatives **32** (*71*) and **33** (*72*), a monoaryl ester **34** of glutarate (*72*), or 2-carboxyanilide **35** (*73*) has been investigated. However, the metal-ion catalysis dominates the overall rate and the introduction of the carboxyl group does not enhance the rate.



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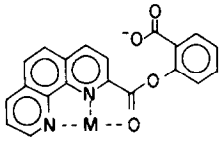

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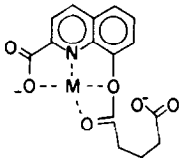

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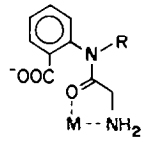

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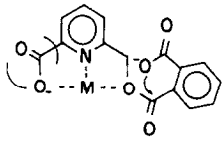
Collaboration by the carboxyl group and the metal ion has been observed in model systems **31** (*63*) and **36** (*48, 74*). The catalytic role of the metal ion is to blockade the inhibitory reverse path in **31** and enhance the leaving ability of the leaving group in **36**. The catalytic roles of the carboxylate group and the metal ion indicated in **31** and **36** are quite different from those of the Glu-270 carboxylate and the Zn(II) ion generally assumed for CPA action (**2–4**). Furthermore, the kinetic data collected for **36** are also compatible with the attack of the metal-bound water molecule, instead of the carboxylate ion, at the ester bond.



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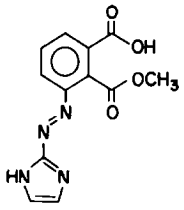
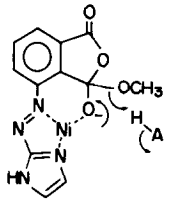
In a model system designed in this laboratory, Ni(II) ion catalyzes the hydrolysis of **37** in water by collaborating with the carboxyl group (*75*). Here, the acidic form of the carboxyl group participates in the cooperative catalysis. Detailed analysis of the kinetic data indicated that the reaction involves rate-controlling breakdown (**38**) of the tetrahedral intermediate. In this reaction, the Ni(II) ion polarizes the carbonyl group, and the carboxylate group acts as a nucleophile, in analogy with the generally proposed roles of the Zn(II) ion and the Glu-270 carboxylate of CPA. However, the Ni(II)-catalyzed hydrolysis of **37** exhibits apparent catalysis by the acidic form, instead of the anionic form, of the intramolecular carboxyl group, in contrast with CPA.

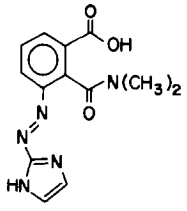


37



38

Amides are the natural substrates of CPA and are chemically much more stable than esters. In this regard, our next step in the design of the CPA model was to achieve the collaboration between the metal ion and the carboxyl group in the hydrolysis of alkyl amides (*76*). For this purpose, metal ion catalysis was investigated for the hydrolysis amide **39**, an analogue of ester **37**. In addition, dimethyl sulfoxide (DMSO) containing 5% (v/v) water was used as the reaction medium, mimicking the microenvironment of the active site of CPA. The metal ion-catalyzed hydrolysis of **37** was also examined in this medium. It has been reported that both the electrostatic and the hydrophobic interactions are facilitated in 95% (v/v) DMSO (*77*). For the formation of CPA–substrate complexes, both the electrostatic interaction between the carboxylate anion of the substrate and the cation of Arg-145 and the hydrophobic interaction between the nonpolar side chain of the substrate and the hydrophobic pocket of the enzyme play important roles.

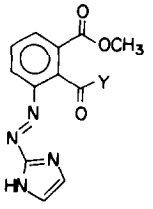


39

In water, the hydrolysis of **39** in the absence of added metal ions proceeds through the apparent participation of the acidic form of the intramolecular carboxyl

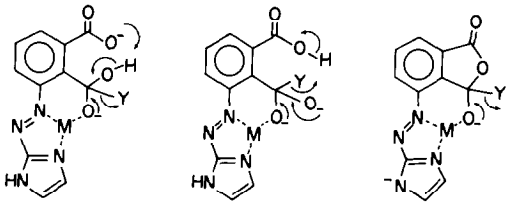
group, as observed with other monoalkyl amides of phthalic acid or maleic acid derivatives. The addition of Cu(II) or Ni(II) ion does not affect the rate of the hydrolysis of **39** in water, although the major portion of **39** is bound to the metal ion under the conditions of the kinetic measurement. Therefore, the hydrolysis of M(II)**39** in water proceeds through the monofunctional catalysis by the intramolecular carboxyl group.

In 95% (v/v) DMSO, the pH of the medium was controlled by changing the ratio of [potassium chloroacetate (CAK)]/[chloroacetic acid (CA)]. Over the pH range of log [CAK]/[CA] = $-1 \sim 1$, the carboxyl group of M(II)**37** and M(II)**39** (M = Cu, Ni) ionizes almost fully. Both the alkyl ester linkage of Ni(II)**37** ($k_0 = 1.3 \times 10^{-4}\ s^{-1}$ at 50°C and log [CAK]/[CA] = 1) and the alkyl amide linkage of Cu(II)**39** ($k_0 = 1.8 \times 10^{-3}\ s^{-1}$ at 50°C and log [CAK]/[CA] = 1) are readily hydrolyzed in 95% (v/v) DMSO. Comparison of the rate data for Cu(II)**39** with those for **39** and Cu(II)**40** and comparison of the rate data for Ni(II)**37** with those for **37** and Ni(II)**41** clearly indicates that both the metal ion and the carboxyl group are the catalytic groups in the hydrolysis of Cu(II)**39** and Ni(II)**37**. Since the carboxyl groups of M(II)**39** and M(II)**37** ionize under the kinetic conditions, the alkyl amide and the alkyl ester linkages are hydrolyzed by the collaboration of the metal ion and the *anionic form* of the carboxyl group.



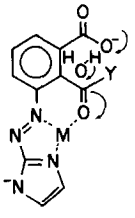
40: Y = $N(CH_3)_2$
41: Y = OCH_3

In the hydrolysis of Ni(II)**37** and Cu(II)**39** in 95% (v/v) DMSO, the intramolecular carboxylate group may or may not act as a nucleophile. Analysis of the kinetic data suggests that **42–44** (Y = OCH_3 or $N(CH_3)_2$) are the possible rate-determining processes. Although it is not possible to choose rigorously the correct mechanism on the basis of the experimental data, consideration of the mechanisms of the hydrolysis of Ni(II)**37**, **37**, and **39** in water and the steric aspects revealed by related systems (*31*) favors the nucleophilic mechanism of **44**.

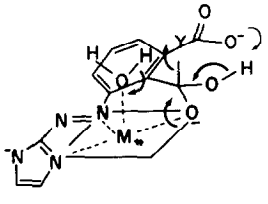


42 **43** **44**

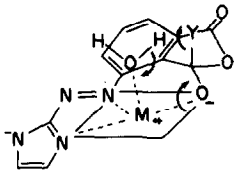
Regardless of the catalytic role of the carboxylate group of Cu(II)**39**, a proton source is needed for the facile expulsion of the amine moiety from the tetrahedral intermediate. This proton may be supplied by the intramolecular carboxyl group (**43**). Here, the proton originates from the water molecule that attacks the acyl carbon in the prior step (**45**). On the other hand, the proton source may be the metal-bound water molecule (**46, 47**).



45



46



47

In the Ni(II)-catalyzed hydrolysis of **37** and the Cu(II)-catalyzed hydrolysis of **39** in 95% (v/v) DMSO, the following catalytic features of CPA are reproduced: (i) Facile hydrolysis of both alkyl ester and alky amide linkages, (ii) collaboration among the metal ion, the carboxyl group, and the reaction medium, (iii) catalytic roles of the metal ion and the carboxyl group similar to those in CPA, and (iv) optimum reactivity attained when the catalytic carboxyl group is in the anionic form.

Both the Ni(II)-catalyzed hydrolysis of ester **37** and the Cu(II)-catalyzed hydrolysis of amide **39** become much faster when the N–H group on the imidazole ring ionizes. In several Zn(II)-metalloenzymes including CPA, the His residue ligating Zn(II) bridges the Zn(II) ion and the carboxylate chain of a nearby Asp or Glu residue (*78*). Thus, the carboxylate–imidazole–Zn(II) triad may be catalytically important, increasing the electron density on the imidazole ring. In this regard, much faster rates observed for the metal-catalyzed hydrolysis of **37** and **39** upon dissociation of the imidazolyl N–H proton may be regarded as the model for the Asp-142/His-69/Zn(II) triad of CPA.

IMPLICATIONS OF CARBOXYPEPTIDASE A MECHANISMS

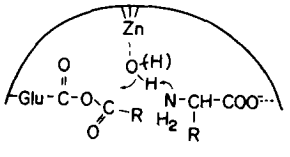
That a certain catalytic feature is manifested by a model is not proof that such a feature is operative in the target enzyme. On the other hand, whether interpretation of data obtained directly with the target enzyme is correctly made

can be evaluated in the light of information derived from model studies. In this regard, the validity of some of mechanistic analysis previously performed on CPA has been tested by using catalytic features revealed by models.

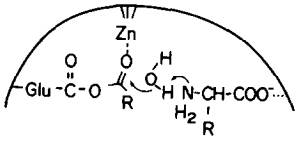
As for the esterase action of CPA, several pieces of experimental data have been obtained in support of the nucleophilic mechanism of Glu-270 (*12, 15–17, 27*). Although some criticisms have been raised against these data (*5,73*), no experimental data have been collected to date directly with CPA in support of the general base role of Glu-270 in the esterase action of the enzyme.

As for the mechanism of the peptidase action of CPA, very few lines of evidence have been obtained directly with the enzyme. The mechanism of **4** has been proposed on the basis of X-ray crystallographic results obtained for the complexes of CPA formed with a pseudo-substrate or inhibitors (*5*). The binding modes of these complexes must be different from those of the dynamic and productive enzyme–substate complexes, and, hence, the crystallographic data do not necessarily provide direct mechanistic evidence. Nevertheless, the general-base-assisted nucleophilic attack by the metal-bound water molecule at an acyl center in **20** can be regarded as a model for the mechanism of **4**.

The kinetic data obtained for the ^{18}O-exchange of *N*-acyl amino acids catalyzed by CPA (*13*) may be regarded as the strongest and, perhaps, the only evidence obtained directly from the catalytic action of CPA in support of the general base role of Glu-270 in the peptidase action. The ^{18}O-exchange rate is much faster in the presence of added amino acids than that of hydroxy acids. This result is readily accounted for by the general base mechanism. However, a model study of **18** and **20** indicates that the breakdown of the anhydride acyl-CPA intermediate may be effectively assisted by the amino acid portion of the substrate (**48**) (*50*). Considering the possible catalysis of anhydride breakdown by amino acids (**48, 49**), the ^{18}O-exchange results are also compatible with the nucleophilic role of Glu-270 (*50*).

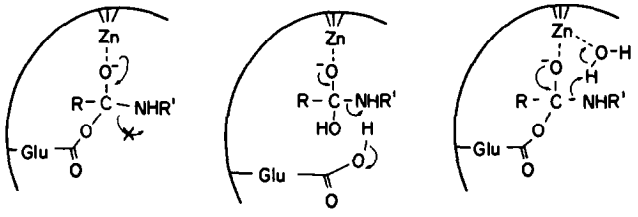


48



49

Cleavage of amide bonds requires a proton donor for the leaving amine. The possibility that the phenol group of Tyr-248 acts as the proton donor in the peptidase action of CPA has been eliminated from the study of the site-directed mutagenetic replacement of Tyr-248 with Phe-248 (*23*). The nucleophilic role of the Glu-270 carboxylate has been questioned (*73*) on the ground that this mechanism apparently does not provide the proton donor (**50**). Instead, if the Glu-270 carboxylate participates as a general base (**3**) in the attack of a water molecule at the acyl center, Glu-270 can act as the general acid catalyst (**51**) during the expulsion of the leaving amine. The results (**47**) obtained with Cu(II)**39** in 95% (v/v) DMSO, however, suggest that the Zn(II)-bound water molecule may act as the proton donor even if the Glu-270 carboxylate behaves as a nucleophile (**52**) (*76*).



50 **51** **52**

From the results of recent X-ray crystallographic studies on CPA, it was suggested that the carbonyl oxygen atom of the scissile amide bond of the substrate would not coordinate to the active-site Zn(II) ion in the productive complex of CPA formed with an amide substrate (*5*). The mechanism of **4** was proposed on this basis. Even if the Zn(II) ion does not bind the carbonyl oxygen, however, it is still possible that the Glu-270 carboxylate makes nucleophilic attack at the carbonyl carbon of the substrate and the Zn(II)-bound water molecule acts as a general acid to protonate the leaving group.

CONCLUSIONS

Catalytic features of CPA reproduced to date by the model systems include collaboration between the metal ion and the anionic form of the carboxyl group in the hydrolysis of alkyl amides and alkyl esters. It is noteworthy that this is achieved by using 95% (v/v) DMSO as the medium.

At present, the rate of amide cleavage achieved by Cu(II)**39** is much slower compared with the enzymatic reaction (k_{cat} = 1~100 s^{-1}). In order to achieve such a high degree of rate acceleration, steric compression between the reaction sites (*31*) as well as the distortion of the amide linkage (*79*) may be added to the model of **39**.

The models would be further elaborated by pursuing the reproduction of other important characteristics of enzymatic catalysis such as specificity and complex formation. The first step toward this goal might be to achieve enantioselectivity toward the amine moiety. Design of models which are capable of both recognizing the structure of CPA substrates and of catalyzing hydrolysis of the substrates very efficiently by employing the catalytic principles of CPA would be the ultimate goal of the model studies of CPA.

Current knowledge on the chemistry of metal ions that act as Lewis acid catalysts in organic reactions is too limited to explain various phenomena observed in model reactions as well as enzymatic systems. For example, it is not explained yet why the bifunctional catalysis in the ester hydrolysis of **37** is achieved with Ni(II) but not with Cu(II) whereas that in the amide hydrolysis of **39** is achieved with Cu(II) but not with Ni(II). In the case of CPA, why the enzyme uses the 5-coordinate Zn(II) ion and why two histidyl nitrogens and one glutamyl oxyen are the binding

ligands of Zn(II) are among the questions to be answered. Further advancement in the model studies would provide clues for these and many other questions.

ACKNOWLEDGMENT

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