mg of **46** in 0.5 mL of solvent (carbon tetrachloride, benzene-d_6, methylene-d_2 chloride, or acetonitrile-d_3) was connected to a vacuum line, degassed by repeating 3 freeze(−196 °C)−pump(10^{-4}−10^{-5} torr)−thaw(0 °C) cycles, and then sealed at 10^{-4}−10^{-5} torr. The NMR tube was placed into a preheated Varian EM-390 90-MHz NMR probe whose temperature was controlled by an EM-3940 Variable Temperature Controller. The concentrations of **45** and **46** were obtained by integrations of methyl signals of **46** and methyl signals of **45** appearing at around 1.9 ppm, and an equilibrium constant was obtained when the ratio of **45** and **46** became constant. The temperature of a probe was calibrated twice before and the ends of the measurements by the standard ethylene glycol or methanol sample. During heating under the conditions employed for rate analyses, any significant side reaction did not take place. The first-order rate constants, k_1 (the retro-1,1-cycloaddition) and k_2 (the 1,1-cycloaddition), were obtained by least-squares treatments of $1/T$ vs. ln $[(KA - B)/(KA_0 - B_0)]$ (A_0 and B_0 are initial concentrations of **45** and **46**, respectively) plots and the equilibrium constant ($K = k_1/k_2$). The first-order rate and equilibrium constants were measured over temperature ranges as follow; for instance in carbon tetrachloride, 20–73 °C for *p*-NO_2, 20.5–48 °C for *p*-CN, 32.8–62.5 °C for *m*-NO_2, 50–76 °C for *p*-Br, 42–62 °C for *p*-Cl, 41.5–62.5 °C for H, 48–67 °C for *p*-CH_3, and 60–71 °C for *p*-OCH_3. The first-order rate and equilibrium constants at 50 °C shown in Tables I and III were the extrapolated values from the linear log k and log K vs. $1/T$ plots. As typical examples, the observed rate and equilibrium constants for the reversible 1,1-cycloaddition of **45e–46e** and **45f–46f** were shown in Table IV.

Acknowledgment. We gratefully acknowledge support of this study by the Grant in Aid for Chemical Research in Development and Utilization of Nitrogen-Organic Resources and for Scientific Research sponsored by the Ministry of Education, Science and Culture, Japan. We also gratefully acknowledge Professors K. N. Houk, R. Huisgen, and A. Padwa for their helpful advice and discussions.

Registry No. 5a, 120-92-3; **6a**, 3197-76-0; **6b**, 5326-50-1; **6c**, 95019-29-7; **7a**, 100188-61-2; **7b**, 1017-23-8; **7c**, 6465-15-2; **8a**, 100188-60-1; **8a**·Na, 73594-32-8; **8b**, 73594-33-9; **8b**·Na, 100188-62-3; **8c**, 73594-34-0; **8c**·Na, 100188-63-4; **10a**, 73594-35-1; **10b**, 73594-36-2; **10c**, 73594-37-3; **11**, 100188-64-5; **12**, 100188-65-6; **13**, 73594-41-9; **13**·Na, 100188-72-5; **14**, 100188-73-6; **15**, 73594-38-4; **16**, 100188-74-7; **17**, 100188-75-8; **18**, 73594-39-5; **18**·Na, 100188-76-9; **19**, 73599-36-7; **20**, 73594-40-8; **21a**, 74457-33-3; **21a**·Na, 74457-34-4; **21b**, 100188-77-0; **21b**·Na, 100189-15-9; **21c**, 100188-78-1; **21c**·Na, 100188-83-8; **21d**, 100188-79-2; **21d**·Na, 100188-84-9; **22b**, 87013-62-5; **23a**, 74457-37-7; **25c**, 87013-65-8; **25d**, 87039-24-5; **26d**, 100188-85-0; **27b**, 100188-80-5; **27d**, 100188-86-1; **28a**, 3420-52-8; **28b**, 28069-36-5; **28c**, 28069-37-6; **28d**, 28069-38-7; **29a**, 90466-88-9; **29a**·Na, 100188-81-6; **29b**, 90466-89-0; **29b**·Na, 100188-82-7; **30a**, 90466-91-4; **30b**, 90466-92-5; **31a**, 90466-94-7; **31b**, 90466-95-8; **32a**, 90466-96-9; **32b**, 3240-29-7; **43a**, 74157-93-0; **43b**, 61752-45-2; **43c**, 100188-87-2; **43d**, 100188-89-4; **43e**, 36597-09-8; **43f**, 100188-91-8; **43g**, 100188-93-0; **43h**, 100188-95-2; **43i**, 100188-97-4; **43j**, 62045-83-4; **43k**, 88299-58-5; **43l**, 73172-57-3; **44a**, 77764-70-6; **44a**·Na, 76620-26-3; **44b**, 77764-70-6; **44b**·Na, 76620-26-3; **44c**, 100188-88-3; **44c**·Na, 100189-02-4; **44d**, 100188-90-7; **44d**·Na, 100189-03-5; **44e**, 76620-32-1; **44e**·Na, 84066-53-5; **44f**, 100188-92-9; **44f**·Na, 100189-10-4; **44g**, 100188-94-1; **44g**·Na, 100189-11-5; **44h**, 100188-96-3; **44h**·Na, 100189-12-6; **44i**, 100188-98-5; **44i**·Na, 100189-04-6; **44j**, 100188-99-6; **44j**·Na, 100189-05-7; **44k**, 100189-00-2; **44k**·Na, 100189-06-8; **44l**, 100189-01-3; **44l**·Na, 100189-07-9; **45b**, 76620-29-6; **46a**, 76633-73-3; **46b**, 76620-30-9; **46c**, 100189-08-0; **46d**, 100189-09-1; **46e**, 76620-31-0; **46f**, 87013-66-9; **46g**, 100189-13-7; **46h**, 100189-14-8; **46i**, 87013-67-0; **46j**, 87013-68-1; **46k**, 87013-69-2; **46l**, 87013-70-5; **47**, 4885-09-0; **48**, 76620-28-5; $BrCH_2CO_2CH_2CH_3$, 105-36-2; $H_3CO_2CCH{=}CHCO_2CH_3$, 624-49-7; $C_6H_5CH(OH)COC_6H_5$, 579-44-2; 1-(carbethoxymethyl)-cyclopentene, 57647-92-4; (carbethoxymethylene)cyclopentane, 1903-22-6; (cyclopenten-1-yl)acetic acid, 21622-08-2; 1-(carbethoxymethyl)-cyclohexene, 4709-59-5; 1-(carbethoxymethyl)cycloheptene, 92599-53-6; (carbethoxymethylene)cycloheptane, 1903-23-7; (cyclohepten-1-yl)acetic acid, 18294-87-6; (cyclohexen-1-yl)acetic acid, 73961-73-6; α-tetralone, 529-34-0; 1-(carbethoxymethyl)-1-hydroxy-1,2,3,4-tetrahydronaphthalene, 91111-41-0; 4-(carbethoxymethyl)-1,2-dihydronaphthalene, 54125-45-0; 4-(carbethoxymethylene)-1,2,3,4-tetrahydronaphthalene, 62677-71-8; (1,2-benzo-1,3-cyclohexadien-3-yl)acetic acid, 4709-55-1; α-(1,2-benzo-1,3-cyclohexadien-3-yl)acetophenone, 100188-66-7; β-(ethoxycarbonyl)tetralone, 6742-26-3; 1-hydroxy-2-(ethoxycarbonyl)-1,2,3,4-tetrahydronaphthalene, 100188-67-8; 3-(ethoxycarbonyl)-1,2-dihydronaphthalene, 100046-58-0; 3-(hydroxymethyl)-1,2-dihydronaphthalene, 100046-59-1; 3-(bromomethyl)-1,2-dihydronaphthalene, 100188-68-9; 3-(1,2-benzo-1,3-cyclohexadien-4-yl)-2-hydroxy-1,2-diphenylpropan-1-one, 100188-69-0; 3-(1,2-benzo-1,3-cyclohexadien-4-yl)-1,2-dihydroxy-1,2-diphenylpropane, 100188-70-3; α-(1,2-benzo-1,3-cyclohexadien-4-yl)acetophenone, 100188-71-4.

Metal Ion Catalysis of Amide Hydrolysis

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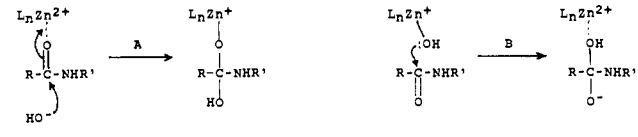
Abstract: Hydrolysis of amides at neutral pH is known to proceed with rate-limiting breakdown of the tetrahedral intermediate (TI). A hypothesis is presented which rationalizes the catalytic effect of metal ions in terms of an acceleration of this step. Coordination of a metal ion to an alkoxide oxygen of the TI greatly diminishes the basicity of the alkoxide oxygen without substantially decreasing its nucleophilicity, resulting in an overall facilitation of expulsion of the leaving nitrogen. General acid catalysis of C–N cleavage by metal-bound water may also be important in certain cases. If TI breakdown is facilitated to such an extent that TI formation becomes partly or wholly rate-limiting, then an additional catalytic benefit of the metal ion in terms of carbonyl activation or metal-hydroxide participation may be realized.

Although the detailed three-dimensional structure of carboxypeptidase A (CPA) has been known for 18 years, the mechanism of peptide bond hydrolysis and the role of the active site zinc remain to this day an open question.[1] Much current research has aimed at distinguishing between two kinetically equivalent pathways leading to the tetrahedral intermediate (TI): (1) the electrophilic activation of the carbonyl moiety by zinc (Scheme IA) and (2) provision of a better nucleophile (Zn–OH mechanism,

Scheme I



Scheme II

Scheme IB). However, this distinction may be academic in view of the substantial evidence which has been accrued indicating that

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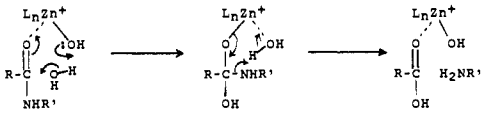
Scheme III



the zinc can become pentacoordinate,[2] raising the possibility of *simultaneous* carbonyl activation and Zn–OH participation,[3,4] the latter involving either direct nucleophilic attack (Scheme IIA) or general base catalysis (Scheme IIB). In addition, in contrast to ester hydrolysis, where the observation of up to a millionfold rate acceleration by metal in model studies[5] has been interpreted in terms of accelerating the rate-limiting TI *formation* step according to Scheme IA and/or IB, the hydrolysis of amides at neutral pH proceeds by way of rate-limiting TI *breakdown* (C–N cleavage),[6] and the nonobservation of significant metal ion catalysis in early model studies[7] appeared to be a consequence of the fact that it is not obvious how C–N cleavage would be facilitated by the metal.[8] Thus, regardless of whether the TI is formed through carbonyl coordination or Zn–OH pathways, the major catalytic phenomenon that must be addressed for the zinc peptidases (and any model amide hydrolyses which *do* exhibit large catalytic effects of metals) is how the involvement of the metal facilitates the otherwise rate-limiting TI breakdown. The purpose here is to clarify this often overlooked, but fundamental question.

Discussion

Amide hydrolysis at neutral pH proceeds according to a rate-limiting TI breakdown mechanism,[6] because, contrary to ester hydrolysis, which involves the relatively facile expulsion of an alkoxide leaving group, amide hydrolysis involves a poor leaving group (formally RNH^-), which must be protonated either prior to or in concert with C–N cleavage. Although catalysis by protons results in a rapid C–N cleavage at low pH, efficient general acid catalysis (GAC) would be required to ensure rapid C–N cleavage at pH 7. It has been previously demonstrated that both external[9] and intramolecular[10] GAC greatly facilitate amide hydrolysis, in some cases resulting in a switch to a rate-limiting TI formation situation. Indeed, early X-ray crystallographic data on CPA appeared to support a GAC role of Tyr-248,[1] though currently the most valid hypothesis[11] appears to be that of Matthews, which invokes GAC by the protonated Glu-270 side chain.[4] Another possibility, which is rarely considered,[8] is that $Zn–OH_2$ could serve as a GAC for C–N cleavage. This is not chemically unreasonable, and an attractive mechanism can be written (Scheme III) that is a simple extension of Scheme IIB. A key point is that if efficient GAC of C–N cleavage results in switching the rate-limiting step to TI formation, *metal ion catalysis of amide hydrolysis should then be observable for the same reason it is in the case of ester hydrolysis*, viz., due to carbonyl-activation and/or Zn–OH participation (Scheme I).

Recently, studies by Groves demonstrated that a system designed to position Cu(II), Ni(II), or Zn(II) above the plane of the amide functionality can be associated with 10^4- to 10^6-fold rate accelerations,[12,13] in contrast to the meager rate enhancements observed in previous models. Groves explained this disparity in terms of the stereoelectronic preference to having the metal held *above* the plane of the amide functionality (for both M–OH and carbonyl activation mechanisms), whereas the earlier models held the metal *in* the plane of the amide, coordinated to a carbonyl oxygen lone pair.[13] Although stereoelectronic considerations are undoubtedly important, they do not address the question of how the presence of the metal has evidently resulted in an acceleration of the normally rate-limiting C–N cleavage step relative to the uncatalyzed system. If, in fact, this acceleration is so large that the hydrolyses are proceeding via a rate-limiting TI formation mechanism (consistent with Groves' ^{18}O-labeling results), then the observed metal ion catalysis can be simply explained according to Scheme I in analogy to ester hydrolysis, without having to invoke any additional (e.g., stereoelectronic) arguments. The big question is why TI breakdown is accelerated. One contributing factor may be that holding the zinc above the plane of the amide linkage allows for an efficient intramolecular GAC of C–N cleavage by $Zn–OH_2$. The pH–rate profile observed by Groves (increasing rate at increasing pH) does not refute this possibility, since the combination of GAC by $Zn–OH_2$ with specific base catalysis by HO^- (in forming an anionic TI) is kinetically indistinguishable from the Zn–OH mechanism proposed by Groves.

Although efficient GAC of C–N cleavage may be a major factor in how the enzyme and certain models achieve metal ion catalysis, an additional, possibly more important mechanism we consider for accelerating C–N cleavage is simply that the incorporation of the metal into the TI (generated through *either* carbonyl-coordination or Zn–OH mechanisms) should inherently accelerate TI breakdown, provided certain conditions are satisfied. Perhaps the best evidence for this notion comes from the elegant experiments by Buckingham and Sargeson[14,15] on kinetically nonlabile Co(III) complexes of glycinamide. In this system, the carbonyl-activation and metal-hydroxide (M–OH) alternatives could be distinguished through oxygen isotope labeling, and both mechanisms were found to exhibit rate enhancements in the range of 10^4 to 10^6.[16] That either of these two mechanisms should be associated with such a large rate enhancement indicates that C–N cleavage has been greatly accelerated, apparently to the point of becoming non-rate-limiting.[14] Why should this be the case? One concern often raised is that the metal in these systems is consumed *stoichiometrically* (the product chelates strongly to the cobalt), so that it is not clear if the conclusions drawn from these results can be extrapolated to *catalytic* reactions. However, since the metal is stoichiometrically coordinated to the substrate as well, it is unlikely that any extra thermodynamic advantage associated

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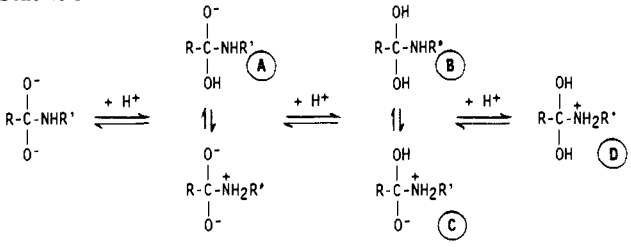
Scheme IV

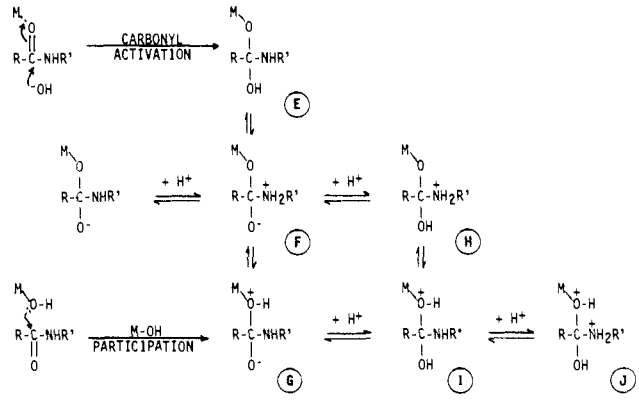


with product chelation can be the major cause of the accelerated C–N cleavage.

An analysis of how we think the presence of a metal in the TI leads to enhanced C–N cleavage rates is based on an argument analogous to that used to rationalize the catalytic advantage of the Zn–OH mechanism, most often cited in regard to carbonic anhydrase and model metal ion mediated hydration reactions. Although Zn–OH is not as good a nucleophile as is HO^- itself, it is a much better nucleophile than is H_2O,[17-19] and the ability of metal ions to enhance deprotonation of water would ensure that at pH 7, [Zn–OH] $\gg$ [HO^-] = 10^{-7} M. The question is whether the increased concentration of [Zn–OH] relative to [HO^-] would more than offset the lower nucleophilicity of Zn–OH relative to HO^-. The answer appears to be yes, since numerous studies on small-molecule model systems have indicated that Zn–OH (pK_a 7–9)[20] retains a surprisingly large fraction of the nucleophilicity of (solvated) HO^- itself (pK_a 15.7), despite a $\sim 10^7$-fold lowering of Brönsted basicity.[18,19,23]

A quantitative assessment of nucleophilicity vs. Brönsted basicity was presented several years ago by Martin[18] in terms of the Swain–Scott treatment of nucleophilic reactivity in protic solvents.[24] Although one would expect to find a linear free-energy relationship for a series of similar[25] nucleophiles, the slope of the log k/k_0 vs. ΔpK_a plot is considerably less than unity, in part because only a fraction of the *thermodynamic* basicity differences are reflected at the transition state as *kinetic* nucleophilicity differences and in part due to HSAB[25] effects. The fact that H_2O and HO^-, which differ in pK_a by 17.4 log units, differ only by 4.2 log units in regard to their S_N2 reaction rates with CH_3Br[24] implies a slope of 0.24 in this case. The slope for the reaction of different hydroxide-containing nucleophilies with propionic anhydride and with CO_2 was found to be 0.25 and 0.18, respectively.[18] With use of 0.24 as a typical value for reactions of hydroxide nucleophilies with electrophilic carbon, it can be shown that a L_nZnOH species with a pK_a 7.4 would be associated with a rate enhancement of about 10^6 compared to unbound hydroxide at pH 7.4.[26] A

Scheme V



corollary of this argument is that for various M–OH systems (including enzymes) it is catalytically disadvantageous to have a pK_a lower than the operational pH, because nucleophilicity would then be diminished without a corresponding increase in the concentration of the active nucleophile. In this regard, the fact that the pK_a for the alleged $Zn–OH_2$ species in carbonic anhydrase[27] and possibly CPA[28] is 7–8 indicates that these enzymes have maximized the catalytic effect obtainable through pK_a lowering. Theoretical evidence for the notion that the interaction of a metal ion with HO^- can greatly diminish its basicity without a corresponding decrease in nucleophilicity has been presented by Pullman.[31]

An inspection of the various possible conjugate acid–base tautomers of the TI for uncatalyzed amide hydrolysis (Scheme IV)[32] reveals that the species most likely to be present at pH 7 are B and D.[33] An examination of the situation with regard to the metal (Scheme V) shows that either carbonyl-activation or Zn–OH participation would lead to tautomeric TI species E–G. Assuming the metal remains stoichiometrically coordinated to the TI oxygen and that the pK_a of the zinc-bound carbinol moiety is about 7–8, the species most likely to be present at pH 7 are E, H, I, and perhaps J. Since TI breakdown can be thought of as a *nucleophilic displacement (of nitrogen) at carbon by the lone pairs on the two oxygens*, the rate of this process should be directly related to the nucleophilicity of the electrons on oxygen in the TI. On the basis of the above discussion of the effect of the metal ion on basicity and nucleophilicity, although E would be less "reactive" than A, it would be more "reactive" than B. Similarly, H would be less reactive than C but more reactive than D. Thus, *based on the same reasoning used to rationalize the validity of the Z–OH mechanism*, the much greater concentration of E and H (relative to I and J) than of A and C (relative to B and D) would more than offset the lesser reactivity. The maximum rate advantage at pH 7 associated with this effect may be about the same as the rate advantage one obtains for the Zn–OH mechanism,

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(25) The hard–soft acid–base (HSAB) theory predicts that Brönsted basicity will parallel nucleophilicity toward "hard" electrophilic centers but will be only partly reflected in "soft" nucleophilicity rate orders.
(26) Referring to a standard state of 1 M for $[Zn^{2+}]_T$ and [ROH], and assuming pK_a values of 15.7 and −1.7 for ROH and ROH_2^+, respectively, then [Zn–OR], [ROH], and [RO^-] are 0.5, 1, and $10^{-8.3}$ M, respectively, at pH 7.4. Using log $k/k_0 = 0.241\Delta pK_a$, one calculates relative rates of 1, 1.3×10^4, and 1.0×10^6 for nucleophilic reactions of RO^-, ROH, and Zn–OR, respectively. Although the rate advantage of Zn–OR over ROH is thus only about 80, several studies[18,19] suggest that the nucleophilicity of H_2O toward certain electrophiles is much less than would be predicted on the basis of the log k/k_0 vs. ΔpK_a plot.
(27) Lindskog, S.; Engberg, P.; Forsman, C.; Ibrahim, S. A.; Jonsson, B.-H.; Simonsson, I.; Tibell, L. *Ann. N.Y. Acad. Sci.* **1984**, *429*, 61. Coleman, J. F. *Ann. N.Y. Acad. Sci.* **1984**, *429*, 26.
(28) It was originally believed[29] that the catalytically critical titration at pH 7–8 represented zinc-bound water, though a few recent studies suggest that the ionization of $Zn–OH_2$ in CPA may occur only at pH >9.[30]
(29) Makinen, M. W.; Kuo, L. C.; Dymowski, J. J.; Jaffer, S. *J. Biol. Chem.* **1979**, *254*, 356. Geoghegan, K. F.; Holmquist, B.; Spilburg, C. A.; Vallee, B. L. *Biochemistry* **1983**, *22*, 1847.
(30) Spratt, T. E.; Sugimoto, T.; Kaiser, E. T. *J. Am. Chem. Soc.* **1983**, *105*, 3679. King, S. W.; Fife, T. H. *Biochemistry* **1983**, *22*, 3603.
(31) Pullman, A. *Ann. N.Y. Acad. Sci.* **1981**, *367*, 340. Pullman, A.; Demoulin, D. *Int. J. Quantum Chem.* **1979**, *16*, 641.
(32) Although it is difficult to say for aliphatic amides whether protonation of the leaving nitrogen occurs in concert with or prior to C–N cleavage (in which case protonation rather than C–N cleavage can be rate limiting), for the sake of this discussion we assumed that whichever is the lower energy pathway in the absence of metals is also the lower energy pathway in the presence of metals. It should be noted that the conclusions from this *comparative* analysis are not dependent on the distinction between prior and in-concert protonation.
(33) It can be assumed that pK_a values for oxygen and nitrogen in the TI will be about three orders of magnitude lower than in ordinary alcohols and amines.

which, as stated above, is considerable.[18,21] The notion that the effect of the metal is to increase the concentration of a normally insignificant, but highly reactive, TI species is supported by the known rapid hydrolysis of trifluoroacetanilides, wherein the electron-withdrawing CF_3 group apparently facilitates the formation of the more "reactive" dianionic (in this case) TI species.[6c,34] An additional advantage associated with the presence of the metal in the TI is that the basicity of the nitrogen in E would be greater than in B, thereby facilitating the protonation required for C–N cleavage. *According to the above hypothesis, the major kinetic advantage of carbonyl-activation or Zn–OH mechanisms pertinent to amide hydrolysis may be not in terms of an effect on the TI-formation step but in terms of the consequences of such on the C–N cleavage step.*

It should be noted that apart from the above argument, one could make a strong case for the hypothesized zinc-catalyzed TI breakdown simply on the basis that the reverse process, i.e., the attack of *unbound* amine on zinc-bound carboxylic acid, should be accelerated by zinc by about the same margin as in other carbonyl activation mechanisms, which, as stated above, is a factor of 10^4 to 10^6. Thus, according to microscopic reversibility (considering the overall process), the acceleration of C–N cleavage by zinc is of equal magnitude.

According to our hypothesis, the criteria that a model must satisfy to exhibit metal ion catalysis of amide hydrolysis are (1) that the metal remain stoichiometrically coordinated as shown in Scheme V throughout the hydrolysis reaction and (2) that the metal does not interact with the leaving nitrogen at the TI stage, where the nitrogen has developed full basic character, since this would inhibit the required protonation (RNH–M cannot be nearly as good a leaving group as RNH_2).[35-37] If the major catalytic effect of the metal ion is manifested in TI breakdown, the question of whether the metal-bound TI is generated through a carbonyl-activation or M–OH route is inconsequential to catalysis. The large effects of metal ions observed by Groves[12,13] can be rationalized in terms of our hypothesis by arguing that the polydentate chelation built into his models ensures that the metal is held in the proper coordination mode to facilitate TI breakdown, encouraging metal–oxygen but preventing metal–nitrogen interaction, thereby achieving the advantages of the Co(III) system discussed above, but without sacrificing catalytic competency. If this is the case, previous findings of a low degree of catalysis for Cu(II), Co(II), Ni(II), and Zn(II) for simpler systems than those employed by Groves probably resulted from the inability of these systems to enforce the correct mode of coordination of the metal ion at the TI.

In regard to the issue of metal–nitrogen interaction, the fact that the hydrolysis of special amide systems (e.g., the strained penicillin lactams) appears to be accelerated rather than inhibited by metal–nitrogen interaction[8] is not contradictory to the above discussion. In the strained lactam systems, C–N cleavage is facile, and coordination of the metal to the nitrogen in the ground state is both (1) encouraged due to strain-induced inhibition of amide resonance and (2) catalytically advantageous because tying up the electrons on nitrogen potentiates the electrophilicity of the carbonyl group, thereby accelerating the rate-limiting TI formation step.

Summary

The above discussion has emphasized that the rate-limiting step in amide hydrolysis in neutral pH regions is breakdown of the TI (C–N cleavage). Thus, the major catalytic role of any metal found to accelerate amide hydrolysis must be a facilitation of the C–N cleavage step. This can result from the ability of metal-bound water to serve as a GAC in protonating the leaving nitrogen and/or from the ability of the metal to facilitate the breakdown of the TI directly. The latter can be viewed in terms of a pK_a lowering effect of the metal in enhancing the nucleophilicity of one of the oxygens in the TI, thereby promoting displacement of nitrogen, or in terms of the reverse of the C–N cleavage step, where coordination of the zinc to the carboxylic acid moiety would activate it toward attack by unbound amine. If the metal accelerates the TI breakdown step to the degree that TI formation becomes partly or wholly rate limiting, then an additional catalytic effect of the metal, involving either carbonyl-activation or M–OH participation, can be realized. In general, one would expect to observe large rate accelerations by metal ions in the hydrolysis of model amide substrates provided (1) that the model binds the metal ion with sufficient affinity to ensure its coordination in the TI (in a mode which discourages its interaction with the leaving nitrogen) and (2) that geometrical restrictions imposed by the model do not preclude stereoelectronic requirements. In the event that $M–OH_2$ does not play a GAC role in the C–N cleavage step, GAC could alternatively be provided by buffer in the case of model reactions or by another active-site group in the case of the metallopeptidases.

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(35) Suh, J.; Baek, D.-J. *Bioorg. Chem.* **1981**, *10*, 266.

(36) A similar situation exists in the hydrolysis of Schiff bases, where it has been observed that metal–nitrogen coordination in the carbinolamine intermediate inhibits C–N cleavage: McDonnell, C. V., Jr.; Michailidis, M. S.; Martin, R. B. *J. Phys. Chem.* **1970**, *74*, 26.

(37) A different situation exists for ester hydrolysis, where, in contrast to C–N cleavage with amides, C–O cleavage occurs without (prior) protonation. Thus, coordination of the metal to the leaving alkoxide oxygen improves leaving group ability in this case: Fife, T. H.; Przystas, T. J. *J. Am. Chem. Soc.* **1982**, *104*, 2251; **1980**, *102*, 7297.