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Metalloenzyme Models. Divalent Metal Ion Catalyzed Hydrolysis of *p*-Nitrophenyl Picolinate in the Presence of Imidazoles and Pyridines Having Hydroxyl Groups in Their Side Chains

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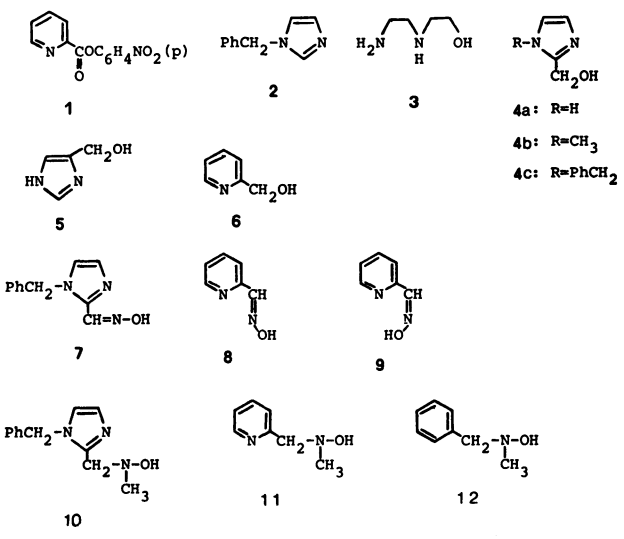
Rate constants for hydrolysis of *p*-nitrophenyl picolinate at 25 °C in the pH range 6.5—8.5 were measured in the absence and presence of divalent metal ions (Ni(II), Zn(II), Co(II), Ca, Mg) and substituted imidazoles or pyridines as ligands having alcoholic hydroxyl groups in their side chains. In the presence of either metal ion or ligand, the rate is slow and the pseudo-first-order rate constant (k_{obsd}) increases linearly in a first-order manner with respect to the concentration of metal ion or ligand until it gives the second-order rate constant, k_M or k_L, respectively. In the presence of both a metal ion (Ni(II) or Zn(II)) and a ligand, rate increase is remarkable for some ligands and the increase in k_{obsd} values constructs saturation curves with respect to increase in either metal ion or ligand concentration. The saturation curves were analyzed based upon rate equations formulated by assuming the formation of 1 : 1 complex of metal ion and ligands as the catalyst, leading to evaluation of the association constant K for complexes and the second-order rate constant k_c for the reaction of complex with substrate. Values of k_{obsd}, k_c, and K are dependent greatly upon the structure of ligands and pH. The ligands complexed with Zn(II) ion appear to be simple but highly active models of hydrolytic metalloenzymes.

Metal ion catalyzed reactions of carboxylic acid derivatives have been extensively investigated in recent years as model reactions of metalloenzymes such as carboxypeptidase A, carbonic anhydrase, and related enzymes.[1-6] It is now well known that a zinc ion is coordinated to two imidazoles and one carboxyl group at the active site of carboxypeptidase A[7-12] and to three imidazole groups at the active site of carbonic anhydrase.[13-16] In these enzymes, the zinc ion is considered to serve as a primary catalytic center for bringing substrate and nucleophile together through formation of a coordination complex, and to activate the substrate carbonyl group toward the action of the nucleophile in carboxypeptidase A or to activate the water molecule in the reversible hydration of carbon dioxide in carbonic anhydrase. In related alkaline phosphatases, the zinc ion appears to activate the serine hydroxyl group.[17,18] Thus it is important for model studies to examine the cooperativity in catalysis between the imidazolyl and hydroxyl groups in the presence of metal ion.

Most of model studies so far conducted are related to intramolecular catalysis, making use of substrates designed to be susceptible to the attack by nucleophile either intramolecularly or intermolecularly through complexation with metal ion.[19] However, there have been known only a few examples of metal ion catalyzed acyl-transfer reactions between coordinated ligands. Breslow *et al.* discovered that the zinc or nickel ion complex of (*E*)-2-pyridinecarbaldehyde oxime, alone[20] or attached to the cyclodextrin moiety,[21] is a remarkably active catalyst for the hydrolysis of esters. Sigman and Jorgensen[22] demonstrated that zinc ion catalyzes the *trans*-esterification between *p*-nitrophenyl picolinate and *N*-(2-hydroxyethyl)ethylenediamine. Very recently, bis(imidazolyl)cyclodextrin[23] and some triimidazolyl derivatives[24] were reported to be effective ligands for the modeling of carbonic anhydrase. However, even in these examples the cocatalysis by imidazolyl and hydroxyl groups has not yet been examined. Furthermore, it should be noted that the catalytic activity of these known complexes is by no means enzymic. Thus complexes more active by several orders should be designed in order to obtain really enzymic artificial enzymes. It has been reported, though with a few examples, that metal-bound imidazolate and hydroxide ions are very reactive nucleophiles.[25]

In our recent communications,[26,27] we reported that *N*-substituted 2-(hydroxymethyl)imidazole-Zn^{2+} ion complexes are much more active catalysts than the above 2-pyridinealdoxime-Zn^{2+} complex for the hydrolysis of *p*-nitrophenyl picolinate. The coexistence of imidazole and hydroxyl groups appears to be essential for the development of catalytic activity, indicating that the system is a simple but good model for the hydrolytic metalloenzymes.

In the present paper, we wish to report a full account of our previous communication[26] as well as results of a further extention including a finding of some more powerful ligands. Activities of ligands **2**—**12** were examined in the presence of divalent metal ions Ni^{2+}, Zn^{2+}, Co^{2+}, Ca^{2+}, and Mg^{2+} for the hydrolysis of *p*-



nitrophenyl picolinate (**1**). These ligands may be classified into two groups: One contains 2-aminomethylamino, imidazolyl, or pyridyl groups as the primary coordination site to bind metal ion, and the other contains alcoholic hydroxyl, aldoxime hydroxyl, or hydroxyamino hydroxyl group as the reacting nucleophile in acyl-transfer reaction.

Results and Discussion

Ligand Hydroxyl Group in Catalysis. Rates of reaction were determined by observing the release of *p*-nitrophenol from the substrate **1** spectrophotometrically. In all cases, under the condition of excess ligand over the substrate, good pseudo-first-order rate constants (k_{obsd}) were obtained up to completion of reaction. Part of the results are shown in Table 1. The table indicates that the uncatalyzed buffer rate constant is $k_0=1.0\times10^{-3}$ min^{-1} at pH 7.05 and 25 °C. In the presence of 1.33×10^{-3} mol dm^{-3} Zn^{2+} ion, the rate increased about 30-fold. This rate increase was little affected by addition of such a ligand as **2** having no hydroxyl group or **12** which is a poor ligand toward Zn^{2+} ion. In the absence of metal ion, the rate enhancement by ligand alone was relatively small, although some of hydroxyl-group bearing ligands showed some enhanced reactivity. At any rate, a large rate enhancement was observed only when ligands with both hydroxyl and neighboring nitrogen groups such as **3**—**11** were used in the presence of either Ni^{2+}, Zn^{2+}, or Co^{2+} ion.

Pseudo-first-order kinetics were also observed under the condition of five molar excess of substrate over the ligand for all the ligands except **3** and **9**, indicating the reaction to occur catalytically. In the case of (*Z*)-2-pyridinecarbaldehyde (**9**), the reaction proceeded in a biphasic manner in which the release of *p*-nitrophenol was initially rapid and then slowed down. Such a biphasic behavior is an evidence for a two-step process involving, in this case, the acylation of the hydroxyl group of catalyst followed by the rate determining deacylation to regenerate the catalyst.[1-3,28] In the case of **3**, as already demonstrated by Sigman and Jorgensen,[22] the second deacylation step was so slow that the reaction ceased in effect at the first acylation step.

The above observation that hydroxyl group is necessary for the enhanced generation and detection of acylated intermediates in **3** and **9**, strongly suggests that catalyses by those ligands with which direct evidence for formation of such acylated intermediates is lacking also occur through the two-step process.

Kinetic Equation. The rates were dependent on both metal ion and ligand concentrations. In the case of *N*-benzylimidazole (**2**) having no hydroxyl group, the k_{obsd} values were found to be given by

$$k_{obsd} = k_o + k_L[L] + k_M[M] + k_{M\cdot L}[L][M], \quad (1)$$

where k_0 is the rate constants due to the buffer, k_L and k_M are the second-order rate constants due to ligand (L) and metal ion (M), respectively, and $k_{M.L}$ is the third-order rate constant for the cocatalysis of metal ion and ligand. The observed values for **2** are $k_L=7.98$ mol^{-1} dm^3 min^{-1}, $k_M=15.3$ mol^{-1} dm^3 min^{-1}, and $k_{M.L}=9.72\times10^3$ mol^{-2} dm^6 min^{-1} at pH 7 and 25 °C.

For other ligands having hydroxyl groups, the rates tended to be saturated with increasing metal ion concentration, as exemplified in Fig. 2 for the case of 1-benzyl-2-(hydroxymethyl)imidazole (**4c**). The figure indicates that in the absence of metal ion, the k_{obsd} values are linear to the ligand concentration with $k_L=1.68$ mol^{-1} dm^3 min^{-1} smaller than the above k_L value for **2**. The saturation curves in Fig. 1 can be analyzed by assuming the formation of 1 : 1 complex of metal ion and ligand according to the kinetic scheme of reactions 2—4 and the set of Eqs. 5—7 for derived rates:

Table 1. Pseudo-first-order rate constants ($k_{obsd}\times10^3$/min^{-1}) for *p*-nitrophenol release from *p*-nitrophenyl picolinate **1** in the presence of metal ion and ligand under the condition of [ligand]$\gg$[**1**][a]

L	M^{2+}					
	None	Ni^{2+}	Zn^{2+}	Co^{2+}	Ca^{2+}	Mg^{2+}
None	1.0	41.1	28.5	23.2	17.1	2.6
2	11.6	—	49.2	—	—	—
3	9.1	134	136	51.3	13.2	9.5
4a	—	—	381[b]	—	—	—
4b	—	—	1008[b]	—	—	—
4c	3.2	1230	2120	268	7.33	13.7
5	—	—	55[b]	—	—	—
6	—	—	242[b]	—	—	—
7	57.4	—	1390	—	75.6	61.9
8	34.1	2590	1570	—	35.4	34.7
9	14.0	525	2530	—	16.6	15.9
10	25.0	718	23500	11700	33.0	40.8
11	18.3	332	14400	4870	20.9	17.8
12	8.8	79.4	24.4	20.3	9.0	8.9

a) Observed at 25 °C, pH 7.05 (0.1 mol dm^{-3} 2,6-lutidine–HNO_3 buffer), $\mu=0.2$ (KNO_3), [L]=[M]=1.33×10^{-3} mol dm^{-3} mol dm^{-3}, and [**1**]=1×10^{-4} mol dm^{-3}. b) [L]=1.0×10^{-3} mol dm^{-3} and [M]=1.33×10^{-3} mol dm^{-3}.

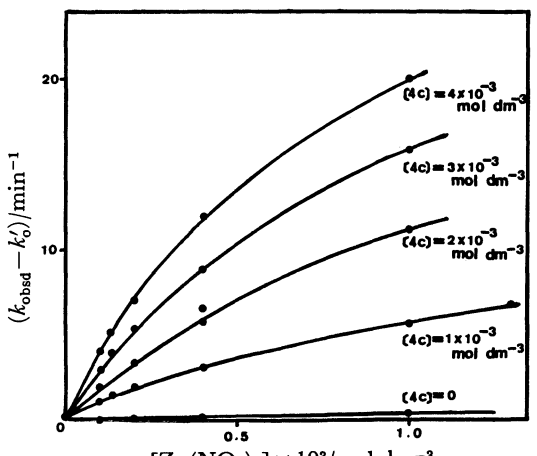


Fig. 1. Plots of pseudo-first-order rate constants for the release of *p*-nitrophenol from **1** as a function of zinc ion concentration; see Table 1 for other reaction conditions.

$$M + L \overset{K}{\rightleftharpoons} \text{complex}, \quad (2)$$

$$S + \text{complex} \xrightarrow{k_c} \text{products}, \quad (3)$$

$$S \xrightarrow{k_o'} \text{products}, \quad (4)$$

$$k_o' = k_o + k_L[L] + k_M[M], \quad (5)$$

$$k_{obsd} = k_o' + \frac{k_c K[L]_T[M]_T}{1+K([L]_T+[M]_T)}, \quad (6)$$

$$\frac{1}{k_{obsd}-k_o'} = \frac{1}{k_c[L]_T} + \frac{1+K[L]_T}{k_c K[L]_T} \cdot \frac{1}{[M]_T}, \quad (7)$$

where L, M, k_O, k_L, and k_M are as defined for Eq. 1, S refers to the substrate, $[L]_T$ and $[M]_T$ are the total concentrations of ligand and metal ion, respectively, K is the association constant between metal ion and ligand and k_C is the catalytic second-order rate constant for the reaction of complex with substrate **1**. A more detailed reaction scheme may involve the complexation between metal ion and substrate, or the formation of ternary complex between the three components (M,L,S). However, such additional complexation equilibria appear to be unimportant, since as mentioned above, the rate was linear to the metal ion concentration in the absence of ligands, indicating that the association constant for the formation of metal ion (Ni^{2+} or Zn^{2+}) and substrate complex is small as compared to that of metal ion and ligand complex.

The above 1 : 1 stoichiometry with the complexation of Eq. 2 was also confirmed for the ligand **4c** by looking at the kinetic version of Job plots shown in Fig. 2. The figure indicates that for both Ni^{2+} and Zn^{2+} ions the plot of k_{obsd} values as a function of mole fraction of **4c** gives a maximum at the mole fraction of 0.5, which corresponds to the 1 : 1 stoichiometry for the kinetically active species.

Saturation curves similar to the one exemplified in Fig. 1 were also obtained for other ligands, although appropriate concentration ranges of metal ion and ligand for kinetics depended on the magnitude of K and k_C values as illustrated in Fig. 3. Based on Eq. 7, three linear plots, *i.e.*, $1/(k_{obsd}-k_o')$ *vs.* $1/[M]_T$, 1/(intercept) *vs.* $[L]_T$, and the slope *vs.* $1/[L]_T$, were obtained for each pair of ligand and metal ion by changing $[M]_T$ and $[L]_T$ and these plots allowed the evaluation of k_C and K. The results of calculation are summarized in Table 2. The table indicates that (*E*)-2-pyridinecarbaldehyde(**8**)-Zn^{2+} ion complex is fairly active in harmony with the activity reported in the literature.[19h,20,21,29] The table also shows that 1-benzyl-2-(*N*-hydroxymethylaminomethyl)imidazole (**10**) and 2-(*N*-hydroxymethylaminomethyl)pyridine (**11**) are more active ligands than **8**. However, for more quantitative comparison, pH dependency of the rate should be examined.

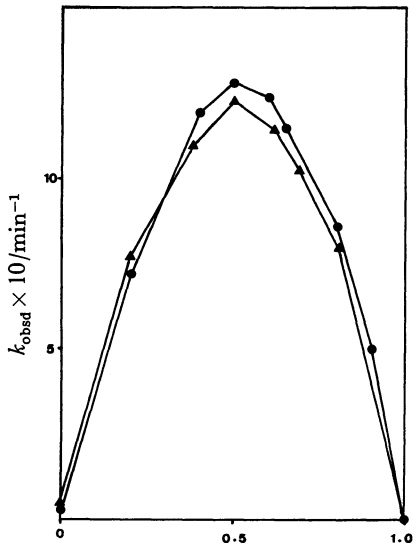


Fig. 2. Job plots for the ligand **4c** and Zn^{2+} and Ni^{2+}. ●: **4c**–Zn^{2+}, $[L]+[M]=2.6\times10^{-3}$ mol dm^{-3}, ▲: **4c**–Ni^{2+}, $[L]+[M]=2\times10^{-3}$ mol dm^{-3}.

pH-Rate Profile. Figure 4 shows plots of log k_{obsd} *vs.* pH for the same reaction conditions as given in Table 1 except for the pH. The figure indicates that both Ni^{2+} and Zn^{2+} ion complexes of **8** yield saturation curves with inflection points at around pH's 6 and 8, respectively. These saturation curves are most likely

Table 2. Association constants (K) and second-order rate constants (k_L and k_C) for *p*-nitrophenol release from *p*-nitrophenyl picolinate (**1**) in the presence of ligand (L) and metal ion (M)[a]

L–M	k_L / mol^{-1} dm^3 min^{-1}[b]	k_C / mol^{-1} dm^3 min^{-1}	K / mol^{-1} dm^3	k_CK or $k_{M\cdot L}$ / mol^{-2} dm^6 min^{-1}
2–Zn^{2+}	7.98	—	—	9.72×10^3
4a–Zn^{2+}	—	2.56×10^3	142	364×10^3
4b–Zn^{2+}	—	8.13×10^3	104	846×10^3
4c–Zn^{2+}	1.68	10.0×10^3	162	1620×10^3
4c–Zn^{2+}	1.68	8.3×10^3	110	913×10^3
5–Zn^{2+}	—	0.31×10^3	95	29.5×10^3
6–Zn^{2+}	—	1.26×10^3	178	224×10^3
8–Zn^{2+}	42.0	4.0×10^3	559	224×10^3
8–Ni^{2+}	—	6.3×10^3	1120	7080×10^3
9–Ni_2^{2+}	12.0	40.0×10^3	44	1760×10^3
10–Zn^{2+}	16.1	27.8×10^3	563	15650×10^3
11–Zn^{2+}	12.1	12.5×10^3	1260	15790×10^3

a) Observed at 25 °C, pH 7.05 (0.1 mol dm^{-3} 2,6-lutidine–HNO_3 buffer), $\mu=0.2$ (KNO_3), $[\mathbf{1}]=1\times10^{-4}$ mol dm^{-3}, and $[L]=[M^{2+}]=1.33\times10^{-3}$ mol dm^{-3}. b) $k_M=15.3$ mol^{-1} dm^3 min^{-1} (see text).

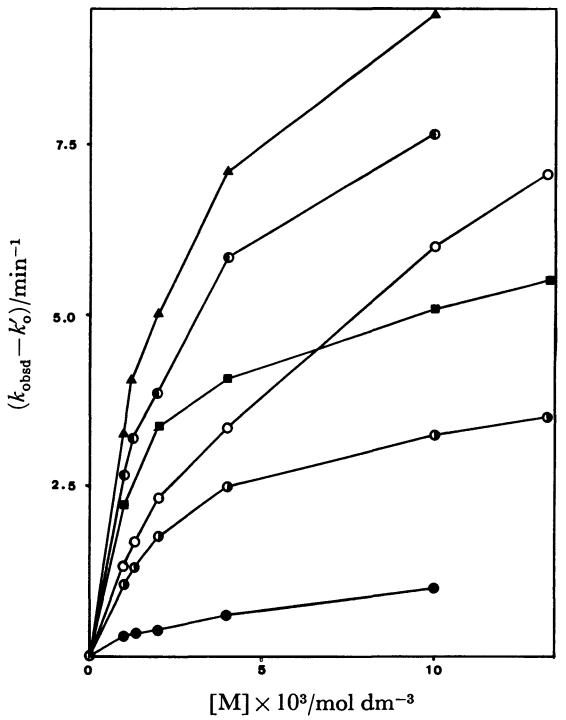


Fig. 3. Plots of $(k_{obsd}-k'_o)$ as a function of metal ion concentration in the presence of the various ligand. ○: **4c**–Zn^{2+}, [**4c**]$=3\times10^{-4}$ mol dm^{-3}, ●: **6**–Zn^{2+}, [**6**]$=1\times10^{-3}$ mol dm^{-3}, ◑: **8**–Zn^{2+}, [**8**]$=1\times10^{-3}$ mol dm^{-3}, ■: **8**–Ni^{2+}, [**8**]$=1\times10^{-3}$ mol dm^{-3}, ▲: **10**–Zn^{2+}, [**10**]$=3\times10^{-4}$ mol dm^{-3}, ◐: **11**–Zn^{2+}, [**11**]$=3\times10^{-4}$ mol dm^{-3}.

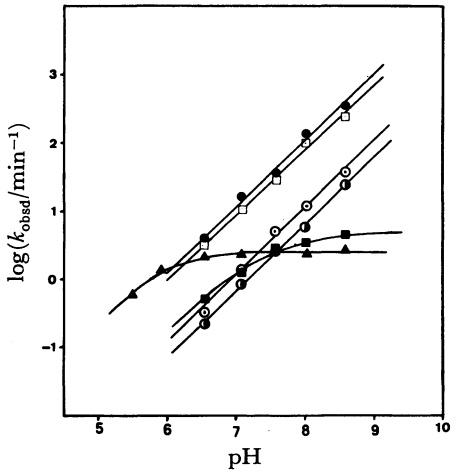


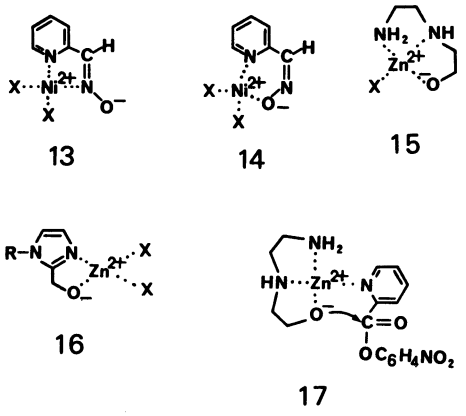
Fig. 4. Plots of k_{obsd}-*vs.* pH for the relase of *p*-nitrophenol from **1** in the presence of 1.33×10^{-3} mol dm^{-3} ligand and 1.33×10^{-3} mol dm^{-3} metal ion; see Table 1 for other reaction conditions. ◑: **4c**–Ni^{2+}, ⊙: **4c**–Zn^{2+}, ■: **8**–Zn^{2+}, ▲: **8**–Ni^{2+}, ●: **10**–Zn^{2+}, □: **11**–Zn^{2+}.

to represent the ionization of hydroxyl group complexed with Ni^{2+} or Zn^{2+} ion. The pK_a 8.6 was assigned to the ionization of hydroxyl group of the latter complex.[20] The lower pH for the ionization of the Ni^{2+} ion complex than that of the Zn^{2+} ion complex suggests that the ligand **8** coordinates to Ni^{2+} ion more tightly than to Zn^{2+} ion, in conformity with the larger K value (1120 mol dm^{-3}) for the Ni^{2+} ion complex than that for the Zn^{2+} ion complex (559 mol dm^{-3}) at pH 7.05, as shown in Table 2.

All the other five complexes, except for those of **8**, gave linear plots with slopes close to unity in the pH range 6.5—8.5. The ligands **10** and **11** having hydroxyamino hydroxyl group are about tenfold more active than **4c** which has a hydroxymethyl group. These linear plots also seem to represent ionization of hydroxyl groups, although their pK_a values must be higher than 8.5. Unfortunately, it was difficult to use higher pH's due to precipitation of metal hydroxide. For this reason, effects of pH on K and k_c values at higher pH's were not examined further. Nevertheless, the data in Fig. 4 together with others may suffice for qualitative discussion on the relationship among catalytic activity, ligand structure, and nature of metal ion.

Effects of Metal Ion and Ligand Structure on the Mechanism of Catalyses. Table 1 indicates that the activity of metal ions at pH 7 in the absence of ligand is in the order $Ni^{2+}>Zn^{2+}>Co^{2+}>Ca^{2+}>Mg^{2+}$. This order may be reasonable if considered on the basis of the ligand affinity of a pyridine base like **1** toward these metal ions. In the absence of metal ion, the activity of ligands is in the order **8**>**10**>**11**>**3**>**4c**. These activities are enhanced 10^2—10^3 times in the presence of Ni^{2+}, Zn^{2+}, or Co^{2+} ion. Ions Ca^{2+} and Mg^{2+} seem to have no activating effect. It is of interest to note that the activating effect of Ni^{2+} ion is somewhat different from those of Zn^{2+} and Co^{2+} ions, in such a manner that in the presence of Ni^{2+} ion the activity order of ligands is **8**>**4c**>**10**>**11**>**3**, which is changed to **10**>**11**>**4c**>**8**>**3** in the presence of Zn^{2+} or Co^{2+} ion. As is clear from Fig. 5, the reactivity order is also pH-dependent. At higher pH's, the difference in reactivity becomes larger between the complexes of **8** and those of the others.

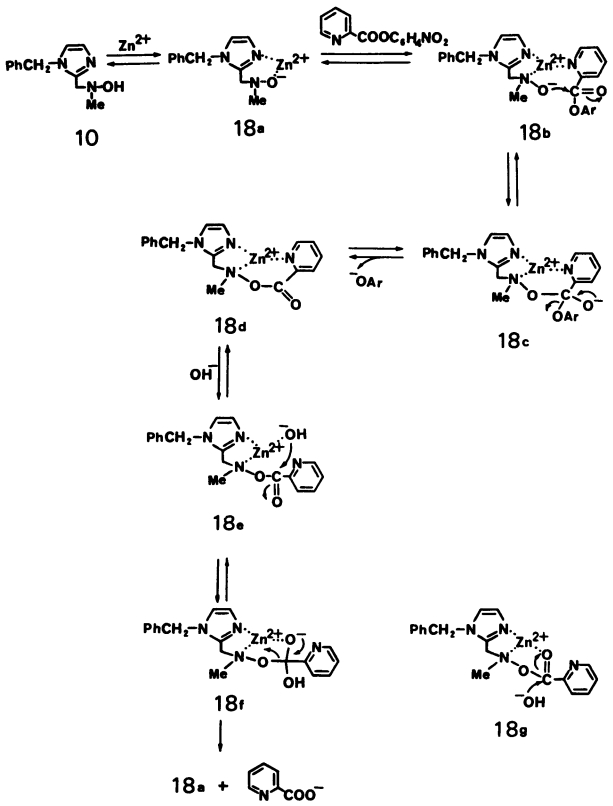
It is most likely that a metal ion activates ligand hydroxyl group by coordination. Comparison of (*E*)- and (*Z*)-oxime **8** and **9** is of interest. The association constants in Table 2 indicate that **8** forms much more stable complexes with Ni^{2+} ion than **9**, presumably because in **8** the two nitrogen atoms are in a coplanar position favorable for chelation with a metal ion, in contrast to **9** (compare the structures **13** and **14**). The activity of complex itself (k_c value), however, is much larger for **9** than for **8**, presumably because the hydroxyl group is bonded to the metal ion much more tightly in **9** (**14**) than in **8** (**13**). Similarly the diamine **3** must form a much stabler complex with Zn^{2+} ion than **4c** (**15** *vs.* **16**), but the resulting activation of hydroxyl group in the former complex is not so large as in the latter complex. Such activation may also be influenced by the nature of nitrogen ligand, such as whether it is aliphatic or aromatic. It is interesting to note with Table 2 that in each pair of **4c**–Zn^{2+} and **6**–Zn^{2+} and **10**–Zn^{2+} and **11**–Zn^{2+}, the imidazole ligands give larger k_c values, whereas the pyridine ligands give larger K values than those of the other. It is also important to note that 2-(hydroxymethyl)imidazole–**4a**–Zn^{2+} ion complex is more active than 4-(hydroxymethyl)-imidazole–**5**–Zn^{2+} ion complex. Moreover, it should be pointed out that the activity of **10**–Zn^{2+} ion complex



is really remarkable as compared to those of known examples in the literature.[20-22]

Sigman and Jorgensen[22] proposed the formation of a reactive ternary complex **17** composed of Zn^{2+} ion, **3** and **1** in the transesterification reaction between **1** and **3**. In this ternary complex the Zn^{2+} ion perturbs the pK_a (estimated to be 8.4) of the hydroxyethyl group of **3** to provide a high concentration of the effective nucleophile, which in turn acts as a template to orient **1** for the intracomplex nucleophilic attack of the ionized hydroxyethyl group on the carbonyl group of **1**. The importance of the formation of the intracomplex for the catalysis was shown by the inability of the **3**–Zn^{2+} ion complex to catalyze the transesterification of *p*-nitrophenyl acetate or *p*-nitrophenyl nicotinate which are unable to form any intracomplex. We also observed a similar substrate specificity in the present systems of **4c**–Zn^{2+} and **10**–Zn^{2+} ion complexes. Thus the formation of the ternary complex **18b**, analogous to **17**, is also conceivable for the catalysis by the **10**–Zn^{2+} ion complex illustrated in Scheme 1. However, as mentioned earlier, such a complex as **18b** should be very low in concentration as compared to the 1 : 1 complex of **18a**. It is rather more reasonable to consider that the Zn^{2+} ion stabilizes the tetrahedral addition intermediate **18c** through which the acylated intermediate **18d** is formed. As already mentioned, the Zn^{2+} ion must also catalyze the deacylation of **18d**. The complex **18e** may explain how the hydroxide ion bound on Zn^{2+} ion facilitates the deacylation through the formation of another tetrahedral intermediate **18f**. Alternatively, the Zn^{2+} ion may act as an electrophile to assist the carbonyl attack by hydroxide ion as illustrated in **18g**, although it is difficult to distinguish between the two mechanisms (**18e** and **18g**) kinetically. Whichever mechanism is true, the breakdown of the tetrahedral intermediate **18f** into products should also be fast for an effective catalysis to occur.

In summary, we have disclosed that the ligands **4c**, **10**, and **11**, when complexed with a metal ion such as Zn^{2+}, Ni^{2+}, or Co^{2+} ion, become highly active toward the hydrolysis of *p*-nitrophenyl picolinate (**1**). The catalysis is most likely to occur through formation of a ternary complex in the transition state or in reactive intermediates. The metal ion in such a complex serves to activate ligand hydroxyl group for nucleophilic attack and to orient the substrate into a favorable position to undergo the reaction. Future interesting subjects are how to incorporate these complexes into more sophisticated systems, such as micellar systems, as active centers of artificial metalloenzymes, although some serious problems still remain to be solved such as how to activate inert substrates.



Scheme 1. Mechanism of hydrolysis of **1** catalyzed by **10**–Zn^{2+} complex.

Experimental

Materials. The water used for kinetic experiments was obtained by distilling deionized water. Buffer reagents, HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid), MES (2-morpholinoethanesulfonic acid), KNO_3, hydrochloric acid, nitric acid, *etc.* were commercial extra pure reagents. Commercially available 2,6-lutidine, *N*-ethylmorpholine, *N*-(2-hydroxyethyl)ethylenediamine (**3**), and 2-(hydroxymethyl)pyridine (**6**) were distilled twice prior to use. Acetonitrile was distilled twice from phosphorus pentaoxide. Commercially available metal salts, $Zn(NO_3)_2 \cdot 6H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$, $Co(NO_3)_2 \cdot 6H_2O$, $Ca(NO_3)_2 \cdot 4H_2O$, and $Mg(NO_3)_2 \cdot 6H_2O$, were used without further purification. *p*-Nitrophenyl picolinate (**1**) was prepared and purified according to the literature procedure.[22] (*E*)-2-Pyridinecarbaldehyde (**8**) was a commercial reagent (Wako Pure Chem.) and was purified by recrystallization from chloroform–hexane. *N*-Benzyl-2-(hydroxymethyl)imidazole (**4c**) was prepared by heating *N*-benzylimidazole and 30% aqueous solution of formaldehyde in an autoclave at 120 °C for 18 h. The resulting mixture was evaporated to dryness and the residue was crystallized after treatment with charcoal from ethyl acetate and white cubes were obtained. These crystals were recrystallized from ethyl acetate and dried: mp 96—97 °C; ^1H NMR ($CDCl_3$) δ=4.60 (2H, s), 4.9 (1H, br s), 5.20 (2H, s), 6.77

(1H, d, J=1.5 Hz), 6.82 (1H, d, J=1.5 Hz), and 7.15—7.30 (5H, m). 1-Benzyl-2-imidazolecarbaldehyde oxime (**7**) was synthesized by slowly adding 1-benzyl-2-imidazolecarbaldehyde and hydroxylamine hydrochloride to a methanol–water (1 : 1 volume ratio) containing sodium carbonate at room temperature. Extraction, drying over magnesium sulfate, concentration *in vacuo*, and column chromatographic separation yielded product **7**: mp 170—171 °C; ^{1}H NMR ($CDCl_3$–$(CD_3)_2SO$) δ=5.55 (2H, s), 7.05 (1H, d, J=1.5 Hz), 7.10 (1H, d, J=1.5 Hz), 7.20—7.35 (5H, m), 8.18 (1H, s), and 11.5 (1H, s). *syn*-Pyridinealdoxime (**9**) was obtained by a photoisomerization of (*E*)-2-pyridinecarbaldehyde oxime (**8**) using a 150 W high-pressure Hg lamp through a Pyrex filter for 30 h. The usual work-up and thin layer chromatographic separation yielded the recovered starting material **8** and (*Z*)-oxime **9**: mp 91—92 °C; ^{1}H NMR ($CDCl_3$) δ=7.3—8.1 (3H, m), 8.68 (1H, s), 8.54—8.65 (1H, m), and 11.25 (1H, br s). 1-Benzyl-2-(*N*-hydroxymethylaminomethyl)imidazole (**10**) was synthesized as follows: *N*-Benzyl-2-chloromethylimidazole hydrochloride, which was obtained by chlorination of **4c** with thionyl chloride, was added to a methanol–water (1 : 5 volume ratio) containing excess amounts of *N*-methylhydroxylamine hydrochloride and sodium carbonate. The usual work-up procedure gave colorless needles of **10**: mp 91—93 °C; ^{1}H NMR ($CDCl_3$) δ=3.30 (1H, s), 4.48 (2H, s), 5.17 (2H, s), 6.88 (1H, d, J=1.5 Hz), and 6.99 (1H, d, J=1.5 Hz). 2-(*N*-Hydroxymethylaminomethyl)pyridine (**11**) was also prepared similarly to **10** and obtained as colorless needles: mp 92—94 °C; ^{1}H NMR δ=2.68 (3H, s), 3.92 (2H, s), 7.00—7.75 (4H, m), 8.0 (1H, br s), and 8.45—8.55 (1H, m). *N*-Hydroxy-*N*-methylbenzylamine (**12**) was obtained by treatment of benzyl chloride with *N*-methylhydroxylamine: mp 41—42 °C; ^{1}H NMR ($CDCl_3$) δ=2.45 (3H, s), 3.66 (2H, s), 7.28 (5H, s), and 8.12 (1H, br s).

Kinetic Measurements. Kinetic runs were generally conducted on a Hitachi 220 spectrophotometer equipped with a thermostated cell compartment. A Hitachi-Horiba pH meter/F-7ss was used for pH determination on buffer and reaction mixtures. The kinetic run was initiated by introducing 10—30 μl of ester stock solution into 3 ml of the buffer solution containing the metal ion and ligand at desired concentrations and maintained at 25 °C. The stock solution contained 1.0—3.0×10^{-2} mol dm^{-3} ester in acetonitrile. The ionic strength was maintained at 0.1 mol dm^{-3} with KNO_3. The rate of hydrolysis of *p*-nitrophenyl picolinate (**1**) was obtained by following the appearance of liberated *p*-nitrophenol at 400 nm. The reaction followed a pseudo-first-order kinetics for at least 3 half-lives. Rate constants and derived kinetic parameters were evaluated by a nonlinear least-squars programs.

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