Hydrolytic Metalloenzyme Models. Micellar Effects on the Activation of the Hydroxyl Groups of *N*-Alkyl-2-(hydroxymethyl)-imidazole Ligands by Cu^{2+} in the Transacylation of *p*-Nitrophenyl Picolinate

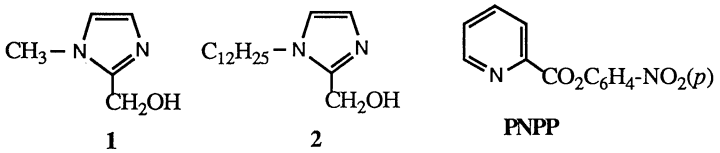
Waichiro TAGAKI,* Kenji OGINO, Osamu TANAKA, Koji MACHIYA, Nobuyuki KASHIHARA, and Toshiharu YOSHIDA
Department of Bioapplied Chemistry, Faculty of Engineering, Osaka City University, Sugimoto-3, Sumiyoshi-ku, Osaka 558
(Received August 24, 1990)

The activation of the hydroxyl groups of two *N*-methyl/dodecyl-2-(hydroxymethyl)imidazole ligands by complexation with Cu^{2+} was investigated kinetically by observing the rates of the release of *p*-nitrophenol in the transacylation of *p*-nitrophenyl picolinate (**PNPP**). The kinetics were carried out in aqueous buffers of pH ranging from 4.5 to 8.0 at 25 °C in the absence of surfactant micelles for a hydrophilic *N*-methyl ligand, and in the presence of micelles for a lipophilic *N*-dodecyl ligand. The kinetic analyses indicated that the 1:1 and 2:1 complexes of the ligand and Cu^{2+} are active nucleophiles with the *N*-methyl and *N*-dodecyl ligand, respectively. The pK_a's of the hydroxyl groups of these complexes were determined to be 7.00 in water and 6.41 in aqueous **CTABr** micelles, respectively, by analyses of the pH-rate profiles. The rate constants, which show the nucleophilic reactivities of ionized hydroxyl anions of complexes toward **PNPP**, were determined to be k_N=1.11×10^4 and 1.25×10^6 mol^{-1} dm^3 s^{-1} with *N*-methyl and *N*-dodecyl ligands, respectively, indicating that the latter micellar complex is 100-times more active than the former non-micellar counterpart.

One of the roles of metal ions in hydrolytic metalloenzymes such as carboxypeptidase A,[1] carbonic anhydrase,[2] or alkaline phosphatase[3] is to activate the substrate as an electrophilic catalysts, stabilizing the negative charges that are formed during the reaction.[4] Another important function of a metal ion is to act as a source of hydroxide ions at neutral pH.[4] It is not uncommon that a metal-bound water molecule ionizes with a pK_a near 7, about 9 units below that of free water, yielding a high fraction of hydroxide ions at neutral pH. Here, a remarkable feature is that such a metal-bound hydroxide ion is a potent nucleophile retaining most of the reactivity of free hydroxide ion.[5,6]

The above-mentioned metal ion functions are quite attractive for designing artificial hydrolytic metalloenzymes. Thus, early in 1972, Sigman and Jorgensen showed that the hydroxyl group of *N*-(2-hydroxyethyl)-ethylenediamine is remarkably activated for the transacylation of *p*-nitrophenyl picolinate (**PNPP**) when complexed with Zn^{2+}.[7] This complex showed the pK_a of the hydroxyl group to be 8.4, much lower than that of the free ligand. More recently, Brown et al. reported that a basic form of the aqua complex of tris(imidazolyl)phosphine and Co^{3+} with pK_a 7.6—7.8 is the active species for the hydrolysis of **PNPP**.[8] Breslow et al. reported that a Zn^{2+} complex of tetraaza-macrocycle is active in the form of hydroxide with a pK_a of 8.7 in the hydrolysis of aryl phosphates.[9] The high reactivities of metal-bound hydroxyl groups with low pK's have also been observed in other recent model studies.[10]

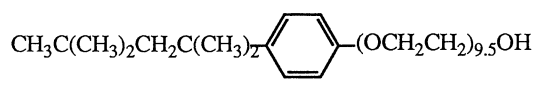
On the other hand, we have been interested in designing micellar models of hydrolytic metalloenzymes.[11] Our search for active ligands has led us to find *N*-alkyl-2-(hydroxymethyl)imidazoles. They were highly active when complexed with bivalent metal ions in the hydrolysis of **PNPP**.[12] Interestingly, the activation was much larger for lipophilic ligands under micellar conditions than for hydrophilic ligands under non-micellar conditions.[13] Recently, there have been an increasing number of studies on the micellar models of hydrolytic metalloenzymes.[13–19] However, most of them were investigated under a particular pH, without dealing with the ionization of a ligand hydroxyl group.



1 **2** **PNPP**

$CH_3(CH_2)_{15}N^+Me_3Br^-$ **CTABr**

$CH_3(CH_2)_{11}OSO_3^-Na^+$ **SDS**



Triton X-100

We wish to report herein the micellar effects on the ionization and reactivity of the hydroxyl group of a Cu^{2+} complex of *N*-dodecyl-2-(hydroxymethyl)imidazole (**2**) ligand, as compared to a non-micellar *N*-methyl counterpart (**1**) in the transacylation of **PNPP**. The surfactants used for micellization were cationic hexadecyltrimethylammonium bromide(**CTABr**), anionic sodium dodecyl sulfate(**SDS**), and non-ionic **Triton X-100**. The ionization of the hydroxyl group of **2** was examined in **CTABr** micelles.

Results

A Survey of Rates at pH 7.03. Ligand **1** was water-soluble, while ligand **2** was hardly soluble in water but, rather, was solubilized by surfactant micelles, so that the kinetics were carried out in plain buffers for the former and in buffered micelles with the latter ligand. The rates of hydrolysis were determined by monitoring the release of *p*-nitrophenol from the substrate **PNPP** spectrophotometrically by using a UV-VIS or a stopped-flow spectrophotometer. In Table 1 are shown the pseudo-first-order rate constants (k_{obsd}) obtained under the conditions of an excess ligand and Cu^{2+} over the substrate at pH 7.03 and 25 °C.

Table 1. Pseudo-First-Order Rate Constants (k_{obsd}) for the Release of *p*-Nitrophenol from **PNPP** at pH 7.03, 25 °C[a)]

Run	System	$k_{obsd}\times10^2/s^{-1}$
1	None	0.00167
2	Cu^{2+}	1.98
3	**1**	0.068
4	**1**+Cu^{2+}	13.8
5	**CTABr**	0.00193
6	**CTABr**+**2**	0.192
7	**CTABr**+Cu^{2+}	3.78
8	**CTABr**+**2**+Cu^{2+}	491

a) In 0.1 mol dm^{-3} 2,6-lutidine-HNO_3 buffer; μ=0.2 (KNO_3), [ligand]=[Cu^{2+}]=1×10^{-4} mol dm^{-3}, [**PNPP**]=5×10^{-5} mol dm^{-3}, [**CTABr**]=1×10^{-2} mol dm^{-3}.

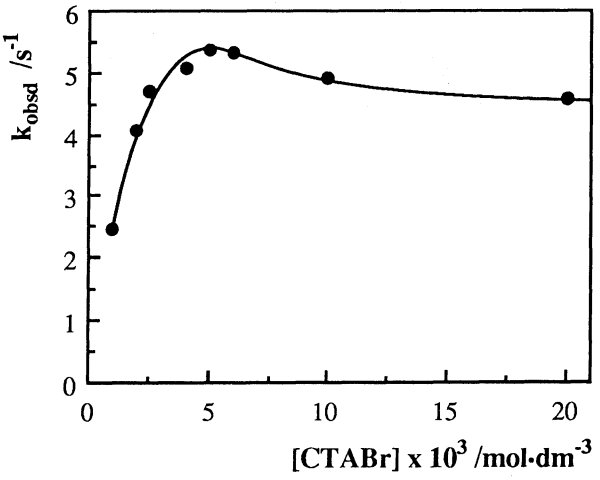


Fig. 1. Effect of **CTABr** concentration on the pseudo-first-order rate constants for the transacylation of **PNPP** at 25 °C, pH 7.03; [**2**]=[Cu^{2+}]=1×10^{-4} mol dm^{-3}, [**PNPP**]=5×10^{-5} mol dm^{-3}.

As indicated, a cationic surfactant **CTABr**, itself, showed almost no rate-enhancing effect (Run 1 vs. 5), although the concentration (1×10^{-2} mol dm^{-3}) used was sufficient to form micelles, i.e larger than the CMC value of about 10^{-3} mol dm^{-3} under the kinetic conditions (see also Fig. 1). The rate enhancement by the ligand alone was also small in each case of **1** under non-micellar (Run 3) and **2** under micellar conditions (Run, 6), as compared to the other cases involving Cu^{2+}. A large rate enhancement of more than 10^3-fold was observed by the addition of 1×10^{-4} mol dm^{-3} of Cu^{2+} (Runs 2 and 7). Even a more remarkable rate acceleration was observed in the coexistence of the ligand and Cu^{2+} (Runs 4 and 8). Thus, a rate enhancement of 4.5×10^5 fold was attained in the presence of 1×10^{-4} mol dm^{-3} each of **2** and Cu^{2+} in **CTABr** micelles (Run 8). As described below, these rate enhancements are dependent on the concentrations of the ligand, Cu^{2+}, and the surfactant, as well as the pH.

Effects of CTABr Concentrations. As shown in Fig. 1, saturation kinetics was observed, i.e. the rates (k_{obsd}) increased with increasing the **CTABr** concentration up to the saturation level. A breaking point in the curve near CMC, usually observed for a micellar reaction,[20,21] could not be detected, simply because of the hardly soluble nature of lipophilic **2** at a low surfactant concentration. The rate saturation of this type can be accounted for as being the saturation of substrate incorporation into the micelles.[20,21] It can be seen that 1×10^{-2} mol dm^{-3} of **CTABr** (Table 1) has sufficient concentration for saturation, and is appropriate as a standard concentration for subsequent experiments.

Reaction Scheme and the Rate Equations. It seems helpful to first explain the reaction schemes and the rate equations used for the analyses of the experimentally observed rates in generalized forms.

As described in the next sections, the rates could be analyzed by assuming a reaction Scheme 1 involving Eqs. 1—3, which is essentially the same as that

$$M + nL \overset{K_M}{\rightleftharpoons} M\text{-}L_n;\ K_M = [M\text{-}L_n]/[M][L]^n \quad (1)$$

$$S + M\text{-}L_n \xrightarrow{k_N'} P \quad (2)$$

$$S \xrightarrow{k_o'} P \quad (3)$$

Scheme 1.

proposed for the Zn^{2+}-catalyzed reactions.[11-13] The scheme indicates that a metal ion (M) forms a complex (M-L_n) with n ligands (L) with an association constant (K_M) (Eq. 1), and that the complex reacts with the substrate (S) bimolecularly in the rate-limiting step with an apparent second-order rate constant (k_N') to afford the products (P) (Eq. 2). The products are also formed through k_o' process without involving an M-L_n complex (Eq. 3). The scheme leads to rate Eqs. 4-11,

$$k_o' = k_o + k_L[L]_T + k_M[M]_T \quad (4)$$

$$\text{Rate} = k_{obsd}[S] = (k_o' + k_N'[\text{M-L}_n]_T)[S] \quad (5)$$

$$n = 1 \qquad k_{obsd} = k_o' + \frac{k_N'K_M[L]_T[M]_T}{1 + K_M([L]_T + [M]_T)} \quad (6)$$

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

$$n = 2 \qquad k_{obsd} = k_o' + \frac{k_N'K_M[L]_T^2[M]_T}{1 + K_M[L]_T^2 + 4K_M[L]_T[M]_T} \quad (8)$$

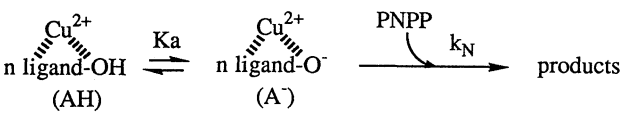
$$\frac{1}{(k_{obsd} - k_o')} = \frac{4}{k_N'[L]_T} + \left(\frac{1}{k_N'} + \frac{1}{k_N'K[L]_T^2}\right) \cdot \frac{1}{[M]_T} \quad (9)$$

$$k_N' = \frac{k_NK_a}{K_a + [H^+]} \quad (10)$$

$$\frac{1}{k_N'} = \frac{1}{k_N} + \frac{1}{k_NK_a} \cdot [H^+] \quad (11)$$

where k_L and k_M are the second-order rate constants due to the ligand and Cu^{2+} alone, and $[L]_T$ and $[M]_T$ are the total concentrations of the ligand and Cu^{2+}, respectively.

An alternative scheme involving a ternary complex, S·M·L_n, may well be considered. The choice of Eq. 2 in Scheme 1 instead of such a ternary complex is simply due to the consideration that the substrate pyridine moiety should be weak as a ligand base in the ground state in aqueous buffers, although it becomes effective upon the formation of an intermediate (see below).



$$[\text{M-L}_n]_T = [AH] + [A^-]$$

Scheme 2.

Eqs. 6 and 8 are approximate equations for 1:1 (n=1) and 2:1 (n=2) complexes of the ligand and metal ion. The validities of these approximations can be confirmed from linear plots of Eqs. 7 and 9. Then, the k_N' and K_M values can be obtained by analyzing the intercepts and slopes. They should be pH-dependent if the active nucleophile is a dissociated complex anion (A^-) as illustrated in Scheme 3. The k_N for the fully dissociated hydroxyl group of the complex and its acid dissociation constants K_a can be obtained based on the relationship between Eqs. 10 and 11.

The Determination of n, k_N, and K_M Values for the Ligands 1 and 2. (a) Job Plots: A convenient method to estimate the above n values is the kinetic version of Job plots,[22] in which the rate constants are plotted as a function of the mole fraction of a ligand or metal ion, keeping their total concentration being constant. The results are shown in Fig. 2. Figure 2 indicates the necessity of the coexistence of the ligand and Cu^{2+} to attain higher rates (k_{obsd}). It also indicates the existence of an optimum γ value to attain a maximum rate. For ligand **1**, the maximum rate is seen at γ=0.5, indicating that the 1:1 complex (n=1) is the active species. For ligand **2**, the rate maxima of three plots are all seen at γ=0.67, indicating that the 2:1 complex (n=2) is the active species in all three kinds of micelles, although the reactivities are variable

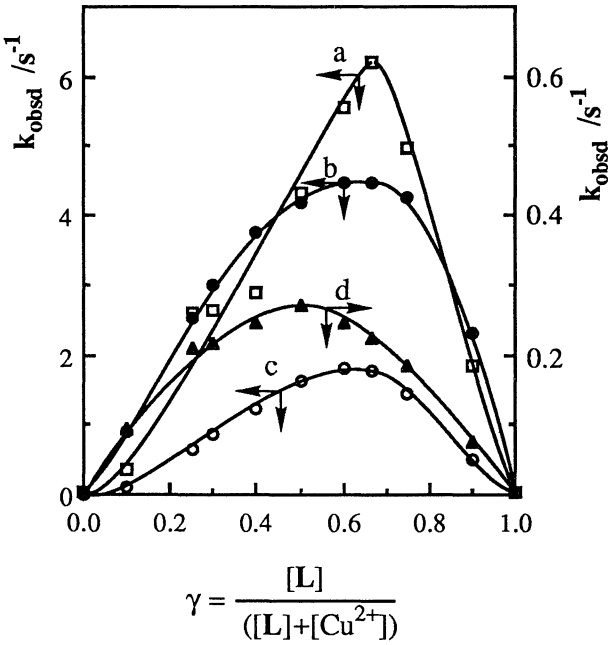


Fig. 2. Job plots for the ligand and Cu^{2+} ion complexation as measured by the rates of transacylation of **PNPP** at 25 °C, pH 7.0: a □, **2**, in [**Triton X-100**]=2×10^{-3} mol dm^{-3}; b ●, **2** in [**CTABr**]=1×10^{-2} mol dm^{-3}; c ○, **2** in [**SDS**]=3×10^{-2} mol dm^{-3}; ([**2**]+[Cu^{2+}])=1×10^{-4} mol dm^{-3}, d ▲, **1** in aqueous buffer, ([**1**]+[Cu^{2+}]=2×10^{-4} mol dm^{-3}, [**PNPP**]=1×10^{-5} mol dm^{-3}.

depending on the micelles.

(b) Saturation Kinetics: As shown in Figs. 3 (**1**) and 4 (**2**), the k_{obsd} values obtained with a constant $[L]_T$ and pH were plotted against Cu^{2+} concentrations ($[M]_T$) to give a saturation curve for each pH. Figures 3 and 4 indicate that the reactivities of **2** under micellar conditions are much higher than those of **1** under non-micellar conditions. Plots of $1/(k_{obsd}-k_o')$ vs. $1/[M]_T$ gave thus straight lines, as shown in Figs. 5 and 6, confirming the relationship between Eqs. 6 and 7 for the ligand **1** (n=1), and Eqs. 8 and 9 for the ligand **2** (n=2). Here, the k_o' values (Eq. 4) were one or two orders smaller than the k_{obsd} values (compare the Runs 2 and 4, or 7 and 8 in Table 1). The intercepts and the slopes of these straight lines allowed us to obtain the k_N' and the K_M values as listed in Tables 2 and 3. The solid lines in Figs. 3 and 4 were calculated by using these k_N' and K_M values and according to Eqs. 6 and 8, again confirming their relationships. The fitting between these calculated solid lines and the experimental k_{obsd} values seems to be satisfactory for most of the pH's

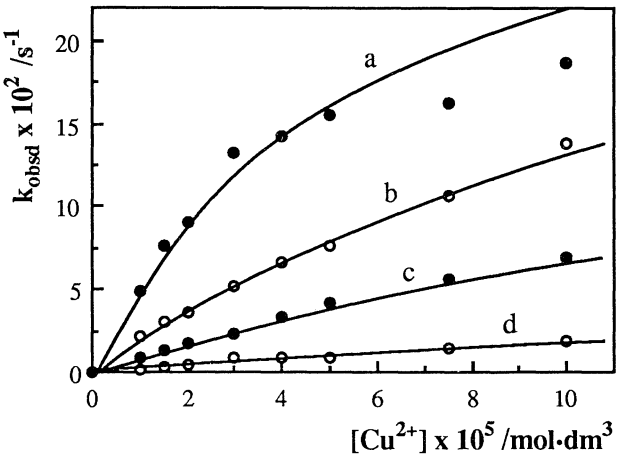


Fig. 3. Pseudo-first-order rate constants for the transacylation of **PNPP** in aqueous buffer at 25 °C as the function of Cu^{2+} concentration: pH's were 7.50(a), 7.00(b), 6.50(c), and 6.00(d); [**1**]=5×10^{-5} mol dm^3, [**PNPP**]=1×10^{-5} mol dm^3.

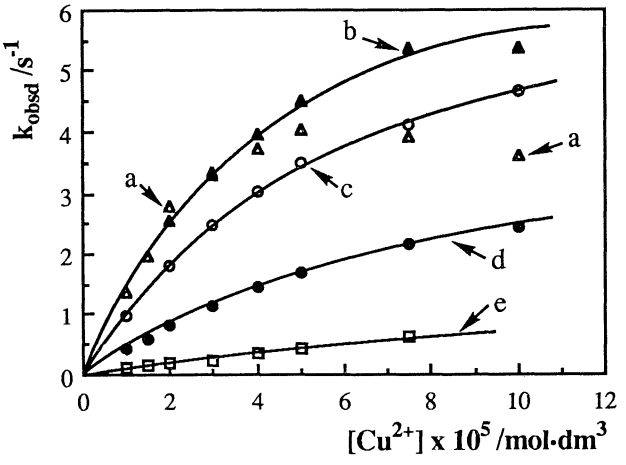


Fig. 4. Pseudo-first-order rate constants for the transacylation of **PNPP** in **CTABr** micelles at 25 °C as the function of Cu^{2+} concentration: pH's were 7.49(a), 7.03(b), 6.50(c), 6.01(d), and 5.51(e); [**2**]= 5×10^{-5} mol dm^3, [**PNPP**]=1×10^{-5} mol dm^3, [**CTABr**]=1×10^{-2} mol dm^3.

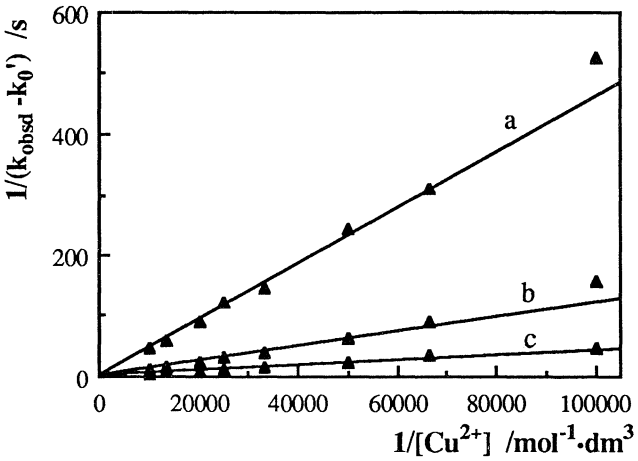


Fig. 5. Plots of $1/(k_{obsd}-k_o')$ vs. $1/[Cu^{2+}]$ for **1-PNPP**: a, pH 6.00; b, pH 6.50; c, pH 7.00.

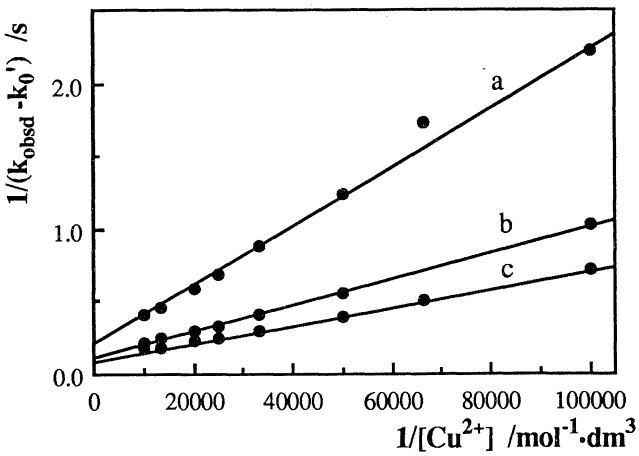


Fig. 6. Plots of $1/(k_{obsd}-k_o')$ vs. $1/[Cu^{2+}]$ for **2-PNPP**: in **CTABr** micelles: a, pH 6.01; b, pH 6.50; c, pH 7.03.

Table 2. pH Dependencies of k_N' and K_M of **1**-Cu^{2+} Complex under Non-micellar Conditions, 25 °C

pH	k_N'/mol^{-1} dm^3 s^{-1}	K_M/mol^{-2} dm^3
4.97	1.05×10^2	2.00×10^3
5.50	3.33×10^2	4.34×10^3
6.00	1.00×10^3	2.16×10^3
6.50	3.16×10^3	4.47×10^3
7.00	7.69×10^3	7.03×10^3
7.50	1.00×10^4	2.07×10^4
8.00	8.85×10^3	2.22×10^4

Table 3. pH Dependencies of k_N' and K_M of **2**-Cu^{2+} Complex under Micellar Conditions, of **CTABr**, 25 °C

pH	k_N'/mol^{-1} dm^3 s^{-1}	K_M/mol^{-2} dm^6
4.46	1.36×10^4	6.46×10^6
4.97	4.46×10^4	6.42×10^6
5.51	1.33×10^5	3.61×10^7
6.01	4.00×10^5	5.75×10^7
6.50	7.62×10^5	6.87×10^7
7.03	8.60×10^5	9.29×10^7
7.49	8.70×10^5	9.09×10^7
8.00	4.04×10^5	5.23×10^7

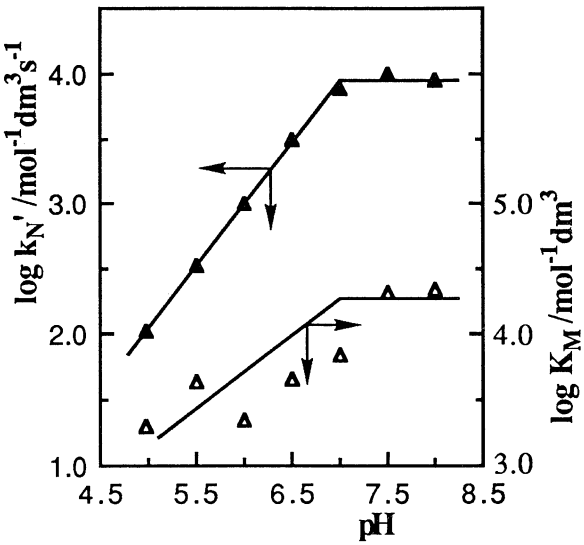


Fig. 7. Plots for log k_N' and log K_M vs. pH for **1**-Cu^{2+} in aqueous buffer.

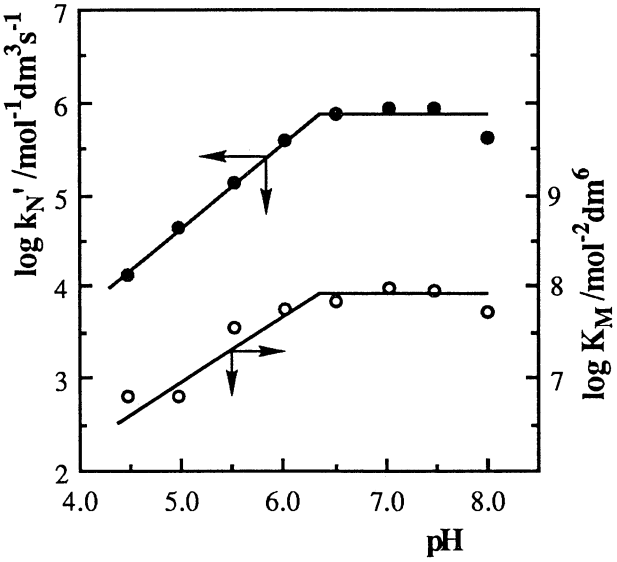


Fig. 8. Plots for log k_N' and log K_M vs. pH for **2**-Cu^{2+} in **CTABr** micelles.

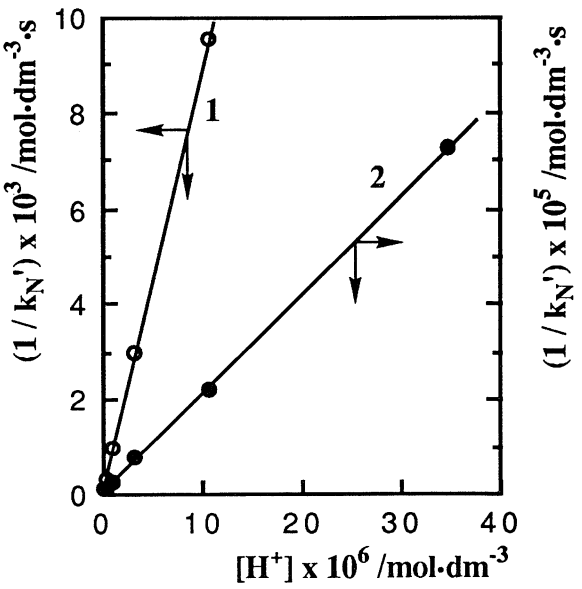


Fig. 9. Plots of $1/k_N'$ vs. $[H^+]$ for the reaction of **PNPP** with Cu^{2+} complexes of **1** and **2**.

examined. However, as can be seen in Figs. 3 and 4, such fitting tends to deviate at higher pH and higher Cu^{2+} concentration, presumably due to the decomposition of active complexes by hydroxide ion as well as the change in the n value.

(c) pH-Rate Profiles: The above k_N' and K_M values were both pH-dependent. As shown in Figs. 7 and 8, the log k_N' values increased linearly with a slope of 1 with increasing pH; they then become pH independent at higher pH. These pH-rate profiles can be accounted for by assuming that the dissociated complex anions (A^- in Scheme 2) are much more active than undissociated complexes (AH). Then, the pK_a's of AH and the k_N values of A^- could be calculated from the plots of $1/k_N'$ vs. $[H^+]$ shown in Fig. 9 (Eq. 11). The results obtained are: $k_N=1.11\times10^4$ mol^{-1} dm^3 s^{-1} and pK_a=7.00 for **1**, and 1.25×10^6 mol^{-1} dm^3 s^{-1} and 6.41 for **2**, respectively. The effects of pH on the log K_M values are qualitatively similar to those on the log k_N' values, i.e. the values increase and then level off with increasing pH. It is reasonable that complexation with a metal ion is stronger with dissociated A^- than with undissociated AH.

Discussion

It has already been established that a bivalent metal-ion catalyzed reaction of an *N*-alkyl-2-(hydroxymethyl)-imidazole with **PNPP** occurs through a two-step process involving the acylation of the 2-hydroxymethyl group, followed by deacylation, although the reaction conditions examined were limited in most cases to those at a particular pH near 7.[11–13] Of these two-steps, the above-mentioned kinetics focused on the acylation step (K_a and k_N in Scheme 2), by carrying

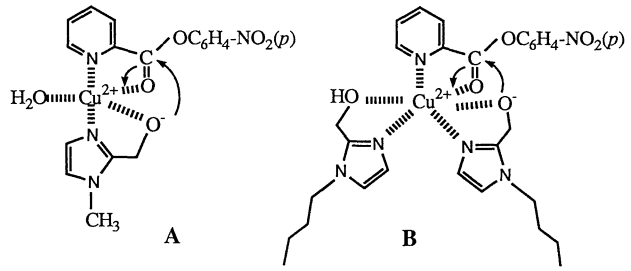


Fig. 10. Proposed structures of ternary complexes of **1** or **2**-Cu^{2+}·**PNPP**.

them out under conditions of excess ligand over the substrate.[23]

These results indicate the following differences between the reactions of non-micellar **1**-Cu^{2+} and micellar **2**-Cu^{2+} complexes: 1) the stoichiometries of complexation, 2) the pK_a's of ligand hydroxyl groups, and 3) the k_N values.

The observed stoichiometry was 1:1 for the former **1**-Cu^{2+}, while 2:1 for the latter **2**-Cu^{2+} complex. Thus, such structures as **A** and **B** are conceivable for the transition states of the two reaction systems, as illustrated in Fig. 10. In both cases, the complexation of **PNPP** with Cu^{2+} involving pyridine nitrogen is presumed to be unimportant at the ground state, but becomes important at the rate-determining step of attacking a ligand-oxido anion nucleophile on the substrate carbonyl carbon to form an addition intermediate.[11,13] The reason for forming a 1:1 complex in a non-micellar system, but a 2:1 complex in a micellar system is not clear. Presumably, the hydration of Cu^{2+} is sufficiently strong to stabilize a 1:1 complex with a hydrophilic ligand in the non-micellar aqueous phase, but not in the micellar phase for a lipophilic ligand; instead, the 2:1 complex becomes more stable. The charge on the micellar surface does not seem to be critical in determining the complex stoichiometry, since the same 2:1 ratio was observed in three different micelles (i.e. anionic, cationic, and non-ionic, Fig. 2).

The pK_a's of the complexes were found to be 7.0 and 6.41 for **1** and **2**, respectively. Thus, for both ligands, a remarkable lowering of pK_a occurred upon complexation with Cu^{2+}, as expected from the previous information.[4] One may notice that the pK_a of **2**-Cu^{2+} is about 0.6 as small as that of **1**-Cu^{2+}. The electron-donating effect as well as the hydrophobic nature to increase pK_a must be larger with the dodecyl than with the methyl group, contrary to observation. However, this reversal of pK_a is not unusual in view of the micellar effect, since it is known that the pK_a of a weak acid, such as phenol, is lowered by 1-2 units when solubilized in cationic micelles, like **CTABr**.[23]

Finally, it is interesting to compare the nucleophilic reactivities of the two systems. At a lower pH, when $[H^+] \gg K_a$, the apparent reactivity is $k_N' = k_N K_a/[H^+]$ (Eq 10). Then, a comparison of the above-mentioned two sets of $k_N K_a$ values indicate that the micellar complex is 428-times more reactive than the non-micellar complex. At a higher pH, when $K_a \gg [H^+]$, k_N' becomes pH independent k_N, that of the dissociated anion (A^-). The ratio of two k_N values indicates that the micellar complex is still 100-times more active than the non-micellar complex. It may be necessary to divide this ratio by a statistical factor of 2 for a fair comparison, since the 2:1 complex has two hydroxyl groups, instead of one in the 1:1 complex. A further correction may be required, since in the micellar reaction the reactants are concentrated in the micellar pseudo-phase so as to enhance the rate of a bimolecular reaction. Bunton et al. proposed a correction factor 0.14 to be multiplied to the experimentally observed second-order rate constant so as to obtain a real micellar rate constant.[20] These two corrections reduce the above 100 to 7. Thus, an apparent large rate enhancement in a micellar system as compared to a non-micellar one seems to be mainly due to a different mode (stoichiometry) of metal ion-ligand complexation as well as to the concentration effect to bring the reactants together in a relatively small volume of a micellar pseudo-phase. However, it should be pointed out that these rate comparisons are still tentative, since the above correction factor (0.14) may not be applied to the present system. The reason is the observation in Fig. 1 that a rate reduction after the maximum is small, as compared to a sharp rate reduction commonly observed for a bimolecular nucleophilic reaction, which requires a correction factor of 0.14 to calculate a real rate constant.

In summary, it has been shown that the Cu^{2+} enhances the apparent reactivity of a coordinated hydroxyl group to a remarkable extent in the neutral pH region by lowering its pK_a. It was further demonstrated that such activation of the hydroxyl group by Cu^{2+} was more pronounced in a micellar system. The obtained information is valuable for any further design of artificial hydrolytic metalloenzymes.

Experimental

Materials. The water used for kinetics was obtained by distilling deionized water twice. Acetonitrile was purified by distillation over P_2O_{10}. Commercially available, 2,6-lutidine, *N*-ethylmorpholine, and acetic acid were purified prior to use by distillation. Commercially available extra-pure $Cu(NO_3)_2 \cdot 6H_2O$ was used without further purification. Other inorganic salts used for buffer preparation were also commercial extra-pure reagents. Hexadecyltrimethylammonium bromide(**CTABr**) and sodium dodecyl sulfate(**SDS**) were recrystallized from acetonitrile before use. Poly(oxyethylene)(9.5) *p*-(1,1,3,3-tetramethylbutyl)phenol (**Triton X-100**, Kishida Chemicals) was used without further purification. The buffers were CH_3COOH–CH_3COONa (pH 4.46—4.97), 2,6-lutidine–HNO_3 (pH 5.50—6.50), and *N*-

ethylmorpholine–HNO_3 (pH 7.03—8.00), and the ionic strength was maintained at 0.2 mol dm^{-3} with NaCl (pH 4.46—4.97) or with KNO_3 (pH 5.50—8.00). *p*-Nitrophenyl picolinate(**PNPP**),[7] *N*-methyl-2-(hydroxymethyl)imidazole,[12b] and *N*-dodecyl-2-(hydroxymethyl)imidazole[13] were prepared and purified according to previous methods. **PNPP** stock solutions for kinetics were prepared in acetonitrile.

Kinetics. Kinetics were carried out according to essentially the same method as reported previously.[11-14] A Hitachi-Horiba pH meter F-8 was used for the pH determination and control. The kinetic runs for slow rates were conducted by using either a Hitachi 220 or 220A spectrophotometer equipped with a thermostated cell compartment. They were initiated by introducing a 10 μl of the **PNPP** stock solution into a 3 ml of a buffer solution containing the desired reagents. Kinetic runs for fast rates were conducted by using an Union Giken RA-401 stopped-flow spectrophotometer equipped with an RA-454 thermobath and an RA-451 computer. The **PNPP** stock solution was diluted by a buffer before use. The reactions were initiated by mixing an equal volume of **PNPP** buffer and the desired reagent solutions. In both slow and fast reactions, the rates were followed by monitoring the release of *p*-nitrophenol at 400 nm (pH 6.5—8.0) or at 320 nm (pH 4.5—6.0). The kinetic runs used for the calculation of rate constants obeyed the pseudo-first-order kinetics for at least 3 half-lives. The absorbance at infinite time (OD_∞) was recorded for each run, and the pseudo-first-order rate constants were obtained by using $k_{obsd}=(2.30/t)\log[(OD_\infty-OD_0)/(OD_\infty-OD_t)]$. The graphical methods for the analyses of these k_{obsd} to obtained k_N', K_M, k_N, and K_a are described in the text (Eqs. 7, 9, and 11).

This research was supported in part by a Grant-in-Aid for Scientific Research No. B 60470097 from the Ministry of Education, Science and Culture.

References

1) W. N. Lipscomb, *Acc. Chem. Res.*, **3**, 81 (1970); D. W. Christianson and W. N. Lipscomb, *ibid.*, **22**, 62 (1989).

2) S. Lindskog, "Adv. Inorg. Biochem.," ed by G. L. Eichhorn and L. G. Marzilli, Elsevier, New York (1982), Vol. 4, Chap. 4, pp. 115—170, S. Lindskog, *Prog. Inorg. Biochem. Biophys.*, **1**, 307 (1986).

3) J. E. Coleman and J. F. Chlebowski, "Adv. Inorg. Biochem.," ed by G. L. Eichhorn and L. G. Marzilli, Elsevier, New York (1979), Vol. 1, Chap. 1, pp. 1—66.

4) A. Fersh, "Enzyme Structure and Mechanism," 2nd ed, W. H. Freeman, New York (1985).

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

6) L. M. Sayre, *J. Am. Chem. Soc.*, **108**, 1632 (1986) and references therein.

7) D. S. Sigman and C. T. Jorgensen, *J. Am. Chem. Soc.*, **94**, 1724 (1972).

8) R. S. Brown, M. Zamkanei, and J. L. Cocho, *J. Am. Chem. Soc.*, **106**, 5222 (1984).

9) S. H. Gelman, R. Petter, and R. Breslow, *J. Am. Chem. Soc.*, **108**, 2388 (1986).

10) J. T. Groves and R. R. Chambers, Jr., *J. Am. Chem. Soc.*, **106**, 630 (1984); T. H. Fife and T. J. Przystas, *ibid.*, **107**, 1041 (1985); J. Suh, A. Han, and B. Chang, *ibid.*, **108**, 1839 (1986); D. R. Jones, L. F. Lindoy, and A. M. Sargeson, *ibid.*, **105**, 7327 (1983); Y. Pocker and N. Janjic, *ibid.*, **111**, 731 (1989).

11) W. Tagaki and K. Ogino, *Top. Curr. Chem.*, **128**, 143 (1985).

12) a) T. Eiki, S. Kawada, K. Matsushima, and M. Mori, and W. Tagaki, *Chem. Lett.*, **1980**, 997; b) K. Ogino, K. Shindo, T. Minami, W. Tagaki, and T. Eiki, *Bull. Chem. Soc. Jpn.*, **56**, 1101 (1983).

13) T. Eiki, M. Mori, S. Kawada, K. Matsushima, and W. Tagaki, *Chem. Lett.*, **1980**, 1431.

14) a) K. Ogino, N. Kashihara, T. Fujita, T. Ueda, T. Isaka, and W. Tagaki, *Chem. Lett.*, **1987**, 1303; b) T. Fujita, H. Minami, K. Ogino, and W. Tagaki, *ibid.*, **1987**, 2289; c) T. Fujita, K. Ogino, and W. Tagaki, *ibid.*, **1988**, 981; d) T. Fujita, Y. Inaba, K. Ogino, and W. Tagaki, *Bull. Chem. Soc. Jpn.*, **61**, 1661 (1988).

15) R. Fornasier, D. Milani, P. Scrimin, and U. Tonellato, *J. Chem. Soc., Perkin Trans. 2*, **1986**, 223; R. Fornasier, P. Scrimin, P. Tecilla, and U. Tonellato, *J. Am. Chem. Soc.*, **111**, 224 (1989).

16) T. Kuwamura, Y. Yano, S. Inokuma, Y. Takenouchi, and H. Tokue, *Chem. Lett.*, **1986**, 1519.

17) F. M. Menger, L. H. Gan, E. Johnson, and D. H. Durst, *J. Am. Chem. Soc.*, **109**, 2800 (1987).

18) L. L. Melhado and C. D. Gutsche, *J. Am. Chem. Soc.*, **100**, 1850 (1978); H.-P. Lau and C. D. Gutsche, *ibid.*, **100**, 1857 (1978); C. D. Gutsche and G. C. Mei, *ibid.*, **107**, 7964 (1985).

19) S. H. Gillman, R. Petter, and R. Breslow, *J. Am. Chem. Soc.*, **108**, 2388 (1986).

20) C. A. Bunton and L. S. Romsted, "The Chemistry of Acid Derivatives," ed by S. Patai, John Wiley & Sons, New York (1977), Part II, Chap. IV.

21) J. H. Fendler, "Membrane Mimetic Chemistry," John Wiley & Sons, New York (1982).

22) A. Martell and S. Chabarek, "Organic Sequestering Agents," Wiley, New York (1989).

23) Preliminary experiments on burst kinetics to use an excess substrate over the ligand indicated that the deacylation step is slow, so that the reaction is not catalytically efficient under the present reaction conditions.