Hydrolytic Metalloenzyme Models. Catalysis in the Hydrolysis of *p*-Nitrophenyl 2-Pyridinecarboxylate by Copper and Zinc Ion Complexes of Anionic Surfactants Having a Functional Imidazole and Hydroxyl Moieties

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(Received March 26, 1992)

Two anionic surfactant-ligands, sodium 2-[1-dodecyl-2,5-bis(dihydroxymethyl)-4-imidazolyl]methylthioethanesulfonate (**1**) and sodium 1-hexadecyl-2-(2-hydroxymethyl-1-imidazolyl)ethyl sulfate (**2**), each having a functional imidazole and a hydroxyl moiety, have been examined regarding their catalytic activities in the hydrolysis of *p*-nitrophenyl 2-pyridinecarboxylate (**PNPP**) in the presence of Zn^{2+} or Cu^{2+} and under co-micellar conditions with surfactants. All of the ligands underwent a remarkable activation of the 2-hydroxymethyl group for transacylation from **PNPP** when complexed with Cu^{2+}. Some interesting differences were observed between the two ligands, **1** and **2**, such as in the modes of complexation and the effects of co-micellar surfactants.

Micellar catalysis has been extensively studied for the past two decades as a model of enzyme catalysis.[1] However, regarding the micellar model of hydrolytic metalloenzymes, studies appear to have begun relatively recently. Examples are the metal-ion complexes of lipophilic imidazoles[2,3] and pyridines[4,5] co-micelled with surfactants. Other metal-ion complexes of the lipophilic derivatives of hydroxylamine,[6] *N*-ammoniocarboxamidate,[7] and *N*-alkylethylenediamine[8] were also examined concerning ester hydrolysis under micellar conditions. In all of these examples, lipophilic ligands are used to form metal-ion complexes; the metal-ion sites of the complexes are presumed to act as polar-head groups of surfactants. However, a few of these lipophilic ligands are functionalized surfactants.[4,7]

In the meantime, we have been continuing our efforts to develop efficient artificial hydrolytic metalloenzymes based on micellar systems. Previously, we found that a lipophilic imidazole ligand having a 2- or 4-hydroxymethyl group is remarkably active when complexed with Zn^{2+} or Cu^{2+} for the hydrolysis of *p*-nitrophenyl 2-pyridinecarboxylate (**PNPP**).[2,3] We further extended our study concerning the catalytic activities of surfactant ligands having a polar head group in addition to a catalytic imidazole moiety, as reported in recent communications.[9] This paper presents a detailed account concerning the catalytic activities of Cu^{2+} and Zn^{2+} complexes of two anionic surfactant-ligands, sodium 2-[1-dodecyl-2,5-bis(dihydroxymethyl)-4-imidazolyl]methylthioethanesulfonate (**1**)[9a] and sodium 1-hexadecyl-2-(2-hydroxymethyl-1-imidazolyl)ethyl sulfate (**2**)[9b] compared with a non-surfactant ligand, 1-dodecyl-2-imidazolylmethanol (**3**), for the hydrolysis of **PNPP** under co-micellar conditions using surfactants: a cationic **HTAB**, an anionic **SDS**, and a nonionic **Triton X-100** as the co-micellar surfactants (Chart 1).

Results and Discussion

Ligands **1**—**5** were prepared as previously reported.[2,3,9a,9b]

Critical Micelle Concentration (CMC) Values. Ligands **1**, **2**, and **5** were confirmed to act as surfactants, as indicated by their cmc values in Table 1. Ligand **2** was hardly soluble in water, but became soluble at higher pHs.

Kinetics. Pseudo-first-order rate constants (k_{obsd}) were obtained by observing the release of *p*-nitrophenol from **PNPP** spectrophotometrically. They were functions of the ligand, metal-ion concentrations, and pHs.

(a) Ligand 1: Micellar Effect : It can be seen in Fig. 1 that the rates (k_{obsd}) increase up to a maximum, then decrease with increasing concentration of the surfactant (ligand) in the presence of a fixed amount of Cu^{2+} or Zn^{2+}. Such a relationship between the k_{obsd} values and the surfactant concentration is known as the rate-enhancing micellar effect, and is explained as being due to an increasing incorporation of reagents (metal-ion and substrate) up to their saturation in a restricted volume of the micellar surface with increasing micelle concentration, but is followed by a dilution of the reagents along with a further increase in the micelle concentration.[10] Regarding Fig. 1, it is also important to notice that the high activity of **1** is due to the presence of a 2-hydroxymethyl group, as can be seen by the low activity of **5**.

Figure 1 also indicates how **1** is activated in the presence of Cu^{2+} and Zn^{2+} at pH 9. In the absence of a

Table 1. cmc Values of Surfactant Ligands at 25°C

Ligands	cmc /mol dm^{-3}	γ/mN m^{-1} (at cmc)	Conditions
1	4.0×10^{-4}	32	Water
2	2.2×10^{-5}	41	pH 9[b]
5	4.0×10^{-4}	32	Water
HTAB	9.2×10^{-4}[a]		
SDS	8.1×10^{-3}[a]		
Triton X-100	3.0×10^{-4}[a]		

a) Ref. 1; b) In borate buffer, 0.1 mol dm^{-3}

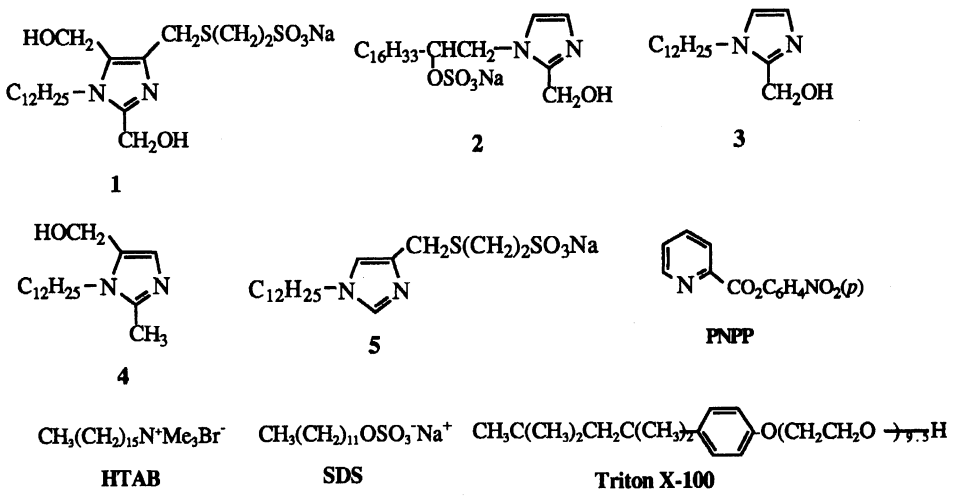


Chart 1. Structures.

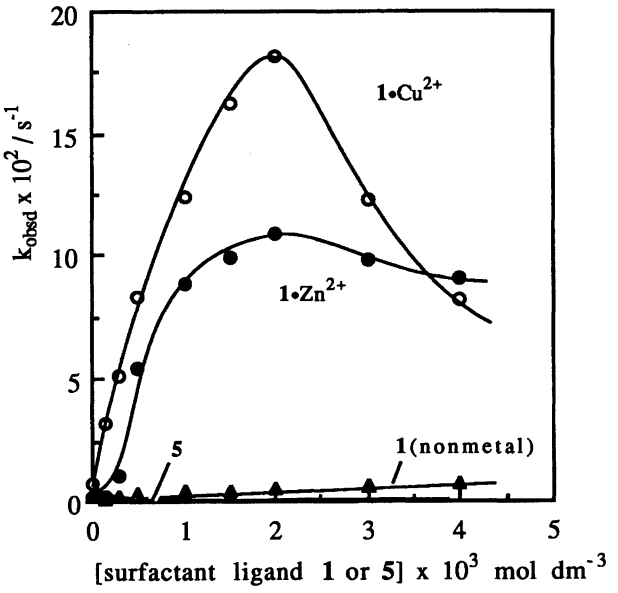


Fig. 1. Plots of k_{obsd} vs. the surfactant concentration for the release of *p*-nitrophenol from **PNPP**, pH 9.0 and 25°C: $[Cu^{2+}]=[Zn^{2+}]=1.3\times10^{-5}$ mol dm^{-3}, $[\mathbf{PNPP}]=5.0\times10^{-5}$ mol dm^{-3}.

metal-ion, **1** showed some activity, being larger than that of **5**; its activity, however, was small, even under micellar conditions (see cmc in Table 1). In contrast, a large activation was observed in the presence of Cu^{2+} and Zn^{2+}, indicating that the active species for catalysis are the ligand/metal-ion complexes. In activation by Zn^{2+} it can be seen that the activation was small, below cmc, but became large above cmc to give a sigmoidal curve, as often observed for micellar reactions, thus indicating that such a ligand/metal-ion complex is much more active in micelles than in non-micellar solutions. It appears that the modes of activation by Cu^{2+} and Zn^{2+} are more or less similar to each other, although the former Cu^{2+} is more active than the latter Zn^{2+}.

(b) Formation and Activation of Metal-Ion Complexes under Co-micellar Conditions : The activation of a ligand by Cu^{2+} was further examined under co-micellar conditions.

(i) Job Plots. The stoichiometry of a kinetically active complex of the ligand and metal ion was determined by a kinetic version of a Job plot.[3e,11] Such Job plots observed for **1** are shown in Fig. 2. In Fig. 2, a rate maximum can be seen at around $\gamma=0.5$ for the plot with **Triton X-100** co-surfactant micelles. For the other two plots with **SDS** and **HTAB**, the positions of

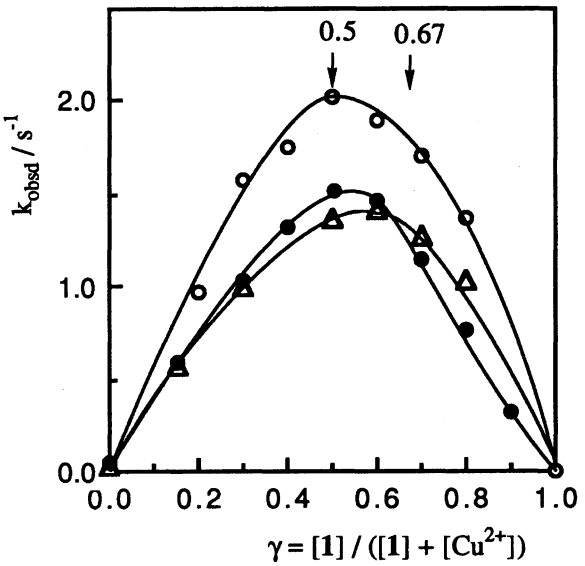


Fig. 2. Job plots for the complexation of ligand **1** and Cu^{2+}, as measured by the rates of the release of *p*-nitrophenol from **PNPP**, pH 7.0 and 25°C: [**1**]+$[Cu^{2+}]=2\times10^{-4}$ mol dm^{-3}, [Co-surfactant]$=1\times10^{-2}$ mol dm^{-3}, $[\mathbf{PNPP}]=2\times10^{-5}$ mol dm^{-3}; ○(**Triton X-100**) ●(**SDS**) △(**HTAB**)

the rate maximum are more or less similar to that with **Triton X-100**. The γ values of 0.5 and 0.67 correspond to the 1:1 and 2:1 molar ratio of the ligand and Cu^{2+}, respectively. Thus, the Job plots suggest that the most active complex of **1** is a 1:1 complex in each of the three co-surfactant micelles. On the other hand, for ligand **2** the Job plots shown in Fig. 3 suggest that a 1:1 complex is more important in an **SDS** micelle, while a 2:1 complex is more important in a **Triton X-100** micelle. For the neutral ligand **3** we previously proposed that a 2:1 complex is the active species in all three kinds of micelles, although the reactivities are variable, depending on the micelles.[3f)]

(ii) Saturation Kinetics: Effects of Co-surfactant on Rate. Although both ligands **1** and **2** have a similar sulfonate and sulfate group as the polar head group of an anionic surfactant, they were found to be widely different regarding their response to a change of the co-surfactant, as shown in Figs. 4 and 5.

Figure 4 indicates that in the presence of a fixed concentration of **1** the rate increases with increasing concentration of Cu^{2+} to give a saturation curve in each of the three co-micelles, presumably involving a 1:1 complex as the major active species. Here, it can be seen that there is essentially no difference between the three co-surfactants in their effects on both the magnitude of the rate and the shape of the saturation curves. In marked contrast, as shown in Fig. 5, there is a big difference for **2** between the curve of **SDS** and those of the other two surfactants, **Triton X-100** and **HTAB**. In the latter **Triton X-100** and **HTAB** co-micelles, it is

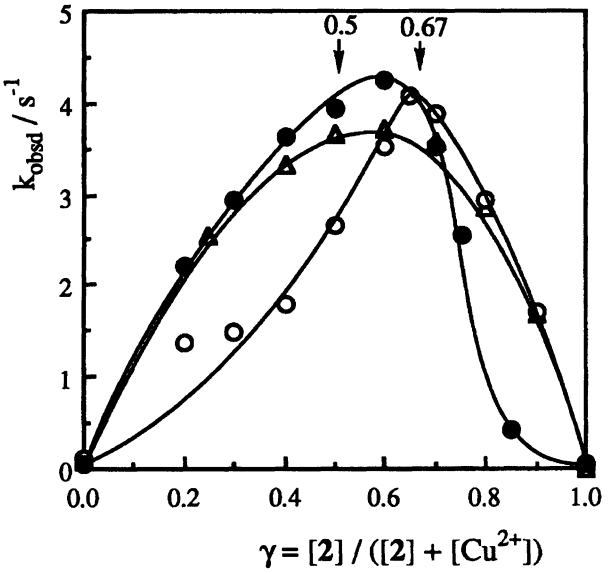


Fig. 3. Job plots for the complexation of ligand **2** and Cu^{2+}, as measured by the rates of the release of *p*-nitrophenol from **PNPP**, pH 7.0 and 25°C: [**2**]+[Cu^{2+}]=2×10^{-4} mol dm^{-3}, [Co-surfactant]=1×10^{-2} mol dm^{-3}, [**PNPP**]=1×10^{-5} mol dm^{-3}; ○(**Triton X-100**) ●(**SDS**) △(**HTAB**)

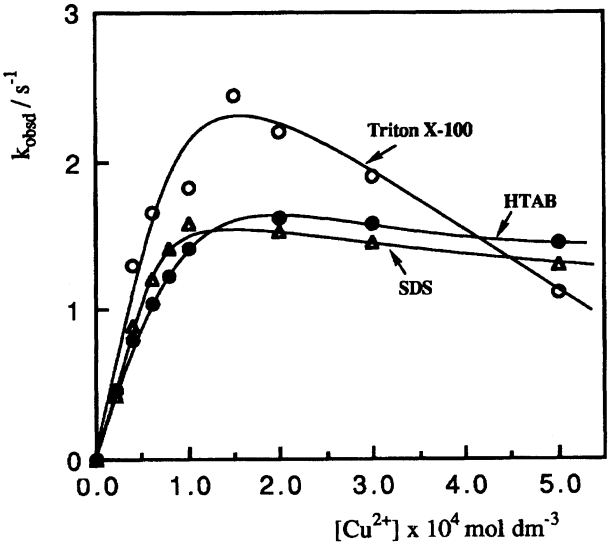


Fig. 4. Effects of the co-surfactant on the **1**·Cu^{2+} catalyzed rates (k_{obsd}) regarding thr release of *p*-nitrophenol from **PNPP**, pH 7 and 25 °C: [**1**]=1×10^{-4}, [**PNPP**]=2×10^{-5}, [surfactant]=1×10^{-2} mol dm^{-3}.

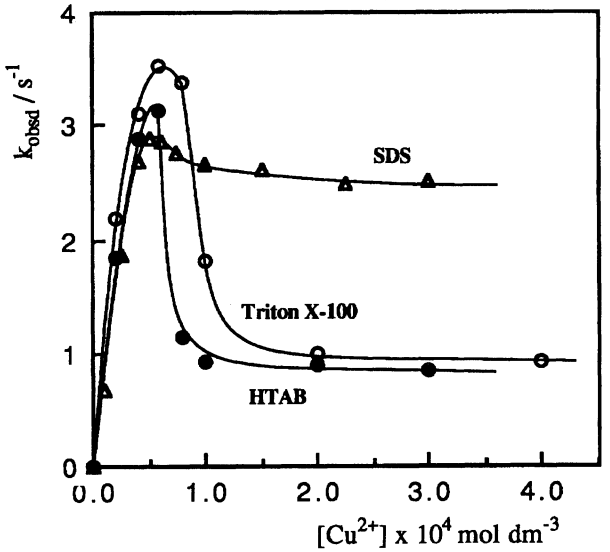


Fig. 5. Effects of the co-surfactant on the **2**·Cu^{2+} catalyzed rates (k_{obsd}) regarding thr release of *p*-nitrophenol from **PNPP**, pH 7 and 25°C: [**2**]=1×10^{-4}, [**PNPP**]=2×10^{-5}, [surfactant]=1×10^{-2} mol dm^{-3}.

conceivable that the 2:1 complex of **2** formed earlier at a lower Cu^{2+} concentration is much more active than the 1:1 complex formed later at a higher Cu^{2+} concentration, thus being in consistent with the Job plot in Fig. 3. On the other hand, the curve of **2** with the **SDS** co-surfactant shows that such a rate drop after the rate maximum is very small. The different response of **1** and **2** to a change of the co-surfactant will be discussed later.

(iii) pH-Rate Profile. In Figs. 4 and 5 it is

reasonable to assume that in **SDS** micelles both **1** and **2** are fully complexed with Cu^{2+} in a 1:1 molar ratio when a Cu^{2+} concentration (2×10^{-4} mol dm^{-3}) exceeds twice that of the ligand (1×10^{-4} mol dm^{-3}). Under such conditions it is possible to determine the ionization constants of the complexed 2-hydroxymethyl group of **1** and **2** by observing their pH-rate profiles.

The pH-rate profiles observed for **1** and **2** are shown in Fig. 6. This figure indicates that in **SDS** co-surfactant micelles the log $(k_{obsd}-k_0')$ increases linearly with increasing pH with a unit slope up to the saturation level for both **1** and **2**. It was also confirmed that there is a linear relationship between $1/(k_{obsd}-k_0')$ and $[H^+]$, as anticipated from the following Eqs. 1, 2, 3, 4, and 5 (see also Scheme 1 and Ref. 3e).

The pseudo-first-order rate constant (k_{obsd}) in Eq. 1 is related to k_N', as in Eq. 2, where k_0' is negligibly small and k_N' is an apparent pH-dependent second-order acylation rate constant. The k_N' is thus related to k_N and K_a, as in Eq. 3, where k_N is the pH-independent acylation rate constant and K_a is the ionization constant of the complexed hydroxyl group. In practice, the k_N and K_a values could be obtained based on the relationship of Eq. 5, where it is assumed that the 1:1 complex concentration is equal to the total concentration of the ligand $[L]_T$ added when $[Cu^{2+}]\gg[L]_T$. The k_N and pK_a thus found, were 1.72×10^4 mol^{-1} dm^3 s^{-1} and 6.84 for ligand **1**, and 4.08×10^4 mol^{-1} dm^3 s^{-1} and 7.01 for **2**, respectively. The k_N value of **2** is larger than that of **1** and is thus in consistent with the results given in Figs. 4 and 5; these values seem to be reasonable when compared to the previous values of the related ligands.[3e,3f] The pK_a values exhibited in the present anionic **SDS** micelle seem to be somewhat higher than those previously observed for the related ligands in a cationic **HTAB** micelle.[3f] This may be reasonable in view of the fact that pK_a of an acid is usually higher in an anionic micelle than in a cationic micelle.[1,10]

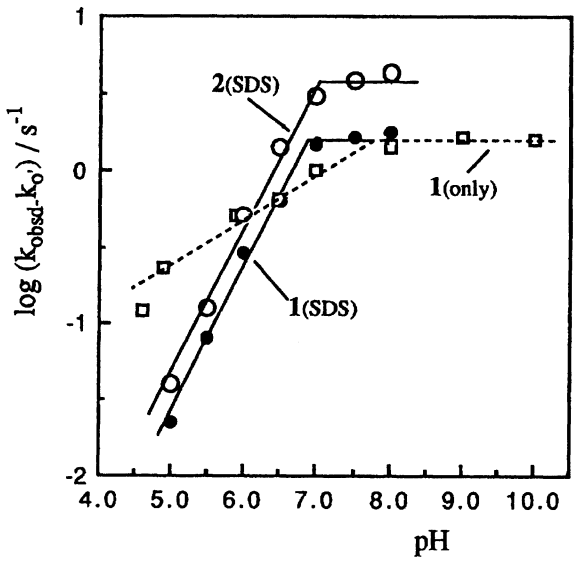


Fig. 6. pH-rate profiles for the release of *p*-nitrophenol from **PNPP**, 25°C: $[\mathbf{PNPP}]=1\times10^{-5}$ mol dm^{-3}, $[\mathbf{1or2}]=1\times10^{-4}$ mol dm^{-3}, $[Cu^{2+}]=3\times10^{-4}$ mol dm^{-3}, $[\mathbf{SDS}]=1\times10^{-2}$ mol dm^{-3}; $[\mathbf{1}]=2.5\times10^{-3}$ mol dm^{-3} and $[Cu^{2+}]=2.5\times10^{-4}$ mol dm^{-3} for **1** only.

$$\text{Rate} = k_{obsd}[S] \quad (1)$$

$$k_{obsd} = k_0' + k_N'[\text{complex}] \quad (2)$$

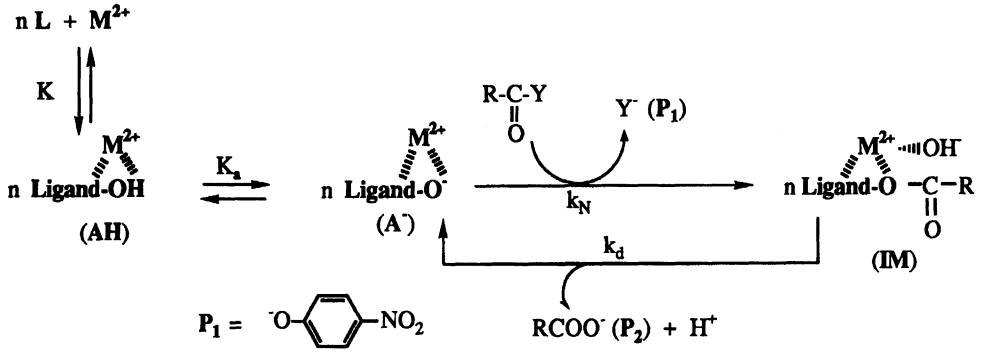
$$k_N' = k_N \cdot K_a / \left(K_a + [H^+]\right) \quad (3)$$

$$1/k_N' = 1/k_N + (1/k_N K_a)\cdot[H^+] \quad (4)$$

$$1/\left(k_{obsd} - k_0'\right) = 1/(k_N[L]_T) + (1/k_N K_a[L]_T)\cdot[H^+]; \quad \text{when } [\text{complex}] = [L]_T \quad (5)$$

Figure 6 also indicates the pH-rate profile of **1** obtained by using only **1** without adding any other co-surfactant. A characteristic feature of the profile is that the slope of the ascending portion is 0.32, much smaller than unity, and the pH (=pK_a) of a breaking point is 7.8, much larger than that in **SDS** co-micelles. The dissociation of a monomer's hydroxyl group to form an anion in a micellar aggregate is not independent of each other, since in such an aggregate any anion formation would suppress any further anion formation, thus raising the average pK_a.

(c) Burst Kinetics. The above-mentioned k_N value is the rate constant for the release of *p*-nitrophenol, i.e. that for the acylation of ligand 2-hydroxymethyl group (Scheme 1). For efficient catalysis to occur, the deacylation to regenerate the catalyst must also be fast, which can be examined by burst-kinetics. The burst-kinetics carried out in **HTAB** cationic micelles under the conditions of 10 molar excess substrate **PNPP** (2.5×10^{-4} mol dm^{-3}) over ligand **1** or **2** (2.5×10^{-5} mol dm^{-3}) are shown in Figs. 7 and 8. As indicated, for each **1** and **2** the release of *p*-nitrophenol occurred in a biphasic manner, known as burst kinetics, where the amount of *p*-nitrophenol released during the initial rapid stage was roughly equal to the amount of ligand employed. It thus seems obvious that the reaction proceeds through the two step acylation/deacylation (Scheme 1). It should be mentioned here that the rate decreased as the reaction proceeded when a sufficient amount of Cu^{2+} was not used, presumably because of product inhibition by 2-pyridinecarboxylate, which inactivates the ligang-Cu^{2+} complex by competitive binding with Cu^{2+}. Although this product inhibition is an interesting subject of future study, Figure 7 indicates that the use of a Cu^{2+} concentration of 7.5—12.5×10^{-5} mol dm^{-3} was sufficient for the observation of a two-step process under the present conditions. Furthermore, both the acylation and deacylation steps for the **1**·Cu^{2+} complex in Fig. 8 are pH-dependent, and the second deacylation step (a zero-order



Scheme 1. Mechanism of **PNPP** hydrolysis catalyzed by a metal ion-ligand complex (A^-) having a nucleophilic hydroxyl group.

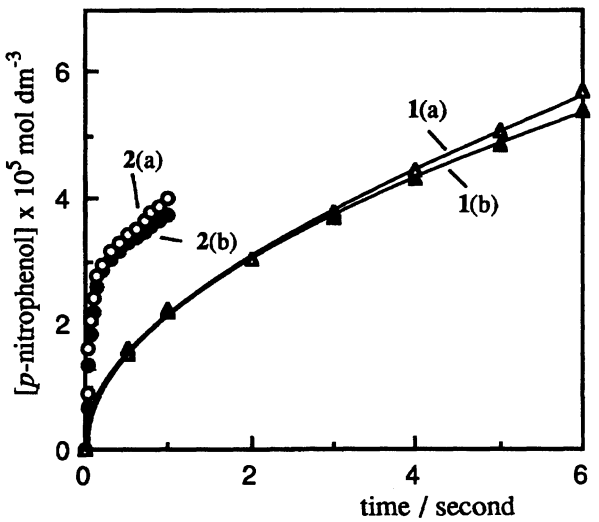


Fig. 7. Burst-kinetics of ligand **1** and **2** regarding the release of *p*-nitrophenol from **PNPP** in **HTAB** co-micelles, 25°C and pH 7: [**HTAB**] = 1×10^{-2} mol dm^{-3}, [**PNPP**] = 2.5×10^{-4} mol dm^{-3}, [1or**2**] = 2.5×10^{-5} mol dm^{-3}; [Cu^{2+}] = 12.5×10^{-5} mol dm^{-3} for **1**(a) and **2**(a), 7.5×10^{-5} mol dm^{-3} for **1**(b) and **2**(b).

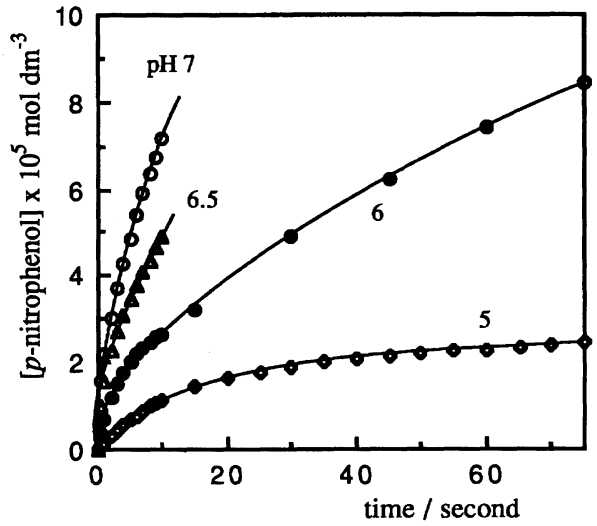


Fig. 8. pH-dependent burst-kinetics of ligand **1** regarding the release of *p*-nitrophenol from **PNPP** in **HTAB** co-micelles, 25°C: [**HTAB**] = 1×10^{-2} mol dm^{-3}, [**PNPP**] = 2.5×10^{-4} mol dm^{-3}, [**1**] = 2.5×10^{-5} mol dm^{-3}, [Cu^{2+}] = 7.5×10^{-5} mol dm^{-3}.

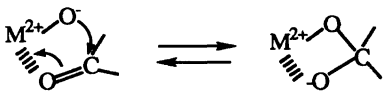
slow step) is reasonably fast, even at pH 6, which indicates that the **1**·Cu^{2+} complex is an efficient catalyst for the hydrolysis of **PNPP** under neutral conditions. It should also be noticed in Fig. 7 that ligand **2** is much more reactive, particularly in the acylation step, than is ligand **1**. It is conceivable for ligand **2** that the major species for catalysis is the more active 2 : 1 complex under the conditions of Fig. 1 regarding the concentrations of the ligand and Cu^{2+} (see also Figs. 4 and 5). Similar burst-kinetics were also observed in **SDS** micelles for both **1** and **2**.

Site of Acylation of 1 in the Presence of Cu^{2+}. As mentioned above, ligand **3** is much more active than is ligand **4**, which shows only negligible activity regarding the release of *p*-nitrophenol from **PNPP** in the presence of Cu^{2+}, thus suggesting that the acylation site of ligand **1** is also the 2-hydroxylmethyl group, rather than the 5-hydroxymethyl group. Further evidence for the site-selective acylation of the 2-hydroxylmethyl group was obtained by an NMR analysis of the products.

A chemical shift of the methylene protons of the 2-hydroxymethyl group of **3** and 5-hydroxylmethyl group of **4** was observed at δ=4.60 and 4.57, respectively. The corresponding methylene protons of **1** were observed at δ=4.61 and 4.60, respectively, although their assignment is not easy at this stage. When treated for a short time (5 second) with excess **PNPP** in the presence of Cu^{2+}, the acylated product(s) of **1** having a 2-pyridylcarbonyl group showed a new singlet peak at δ=5.48, together with a much diminished methylene peak δ=4.61 and an unchanged peak δ=4.60. The δ=4.60 methylene peak of **3** also changed to δ=5.52 upon acylation with the 2-pyridylcarbonyl group; the diacetate of **1** showed methylene peaks at δ=5.21 and 5.16. These

results indicate that the acylation of **1** with **PNPP** in the presence of Cu^{2+} takes place predominantly at the 2-hydroxymethyl group.

Mechanism of Catalysis. The mechanism outlined in Scheme 1 involving the acylation and deacylation steps should be discussed in more detail. First, in the case of **1**, the mechanism is likely to be depicted as in Fig. 9, where a 1:1 complex (Cu^{2+}·**1**$^-$, **A**$^-$) is assumed to be the catalytic species. The catalyst **A**$^-$ forms a ternary complex **a** on a micellar surface which should be more favored than in the aqueous phase; the formation of an additional tetrahedral addition intermediate **b**, followed by an acylated intermediate **c**, would be assisted by chelation with a pyridine moiety with a fast rate (k_N[**b**]). The deacylation would proceed similarly, i.e. the incorporation of a hydroxide ion from the aqueous phase on the complex-Cu^{2+} facilitates the deacylation step. In Fig. 9, the following mechanism (Eq. 6) is considered based on proposals found in the literature[12] in which a metal ion is presumed to act bifunctionally as an acid and a base.



(6)

An interesting observation is the different response of **1** and **2** to a change of the co-surfactant (Figs. 4 and 5). As already mentioned above, it is reasonable to consider that an initially formed 2:1 complex of **2** is transformed into a 1:1 complex with increasing Cu^{2+} concentration, and that the activity of the latter complex sharply decreases in **Triton X-100** or **HTAB** micelles, as compared to that of the former 2:1 complex; such an activity drop is small in **SDS** micelles. What is the origin of such a difference between **1** and **2** ? As shown in Fig. 10, it is reasonable to consider structure **a** for **2**, a 2:1 complex, which was already proposed for the 2:1 complex of **3**.[2,3a,3e] Then, **a** would be readily transformed to a 1:1 complex **b** by the formation of a salt bridge between Cu^{2+} and the two sulfate groups with increasing Cu^{2+} concentration. Here, intramolecular chelation of the sulfate group with Cu^{2+} is possible, like in the 1:1 complex of **1** (Fig. 9). Thus, with respect to the ligand composition, the 1:1 complex of **1** is a monomer, while that of **2** is a dimer. It seems important to notice that such a salt bridge in **b** should be the most stable in nonionic **Triton X-100** co-micelles. In cationic **HTAB** co-micelles, a large and monovalent trimethylalkylammonium cation is a much weaker cation than a small, divalent Cu^{2+} cation in salt formation with a sulfate anion. Thus the formation of a salt bridge in **b** is also favored in **HTAB** co-micelles. On the contrary, the sulfate head group of **SDS** co-micelles may well compete with the sulfate group of **2** for the salt formation with Cu^{2+}, obstructing the formation of the salt bridge. Presumably, a more favored coordination structure for catalysis in **a** is disturbed in **b** indirectly by such salt formation. Additionally, the locations of the active sites of the Cu^{2+}-ligand complexes on the micellar surface and/or the difference in the strength of the interaction of the active sites with the polar-head groups of surfactants may affect their activities, as in the following order: **3** in **Triton X−100**≈**2** in all three micelles >**3** in **HTAB**>**3** in **SDS**≈**1** in all three micelles.[3f,9e]

In conclusion, it can be summarized that (a) the 2-hydroxylmethyl groups of the lipophilic 1-alkylimidazole derivatives exhibit enhanced nucleophilic reactivity under neutral conditions when complexed with Cu^{2+} in surfactant co-micelles, and (b) the kinetic response of such a complex to a change of the co-surfactant depends on a combination of ligand and co-surfactant structures, which occurs very sharply under certain conditions. Such information as the latter (b) is so far very limited that it deserves to be the subject of further investigations.

Experimental

Materials. The water used for the kinetics was obtained by distilling deionized water twice. Acetonitrile was purified by distillation over P_4O_{10}. Commercially available 2,6-lutidine, *N*-ethylmorpholine, and acetic acid were purified prior to use by distillation. Commercially available extra-pure $Cu(NO_3)_2$·$6H_2O$ and $Zn(NO_3)_2$·$6H_2O$ were used without any further purification. Other inorganic salts used for buffers were also commercial extra-pure reagents. Other organic solvents were purified according to the standard procedures. Hexadecyltrimethylammonium bromide (**HTAB**) 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 any further purification. The buffers were CH_3COOH/CH_3COONa (pH 4.50—5.50), 2,6-lutidine/HNO_3 (pH 5.50—6.50), *N*-ethylmorpholine/HNO_3 (pH 7.00—8.00), and H_3BO_3/NaOH (pH 8—10); the ionic strength was maintained at 0.2 mol dm^{-3} with NaCl or KNO_3. The ligands **1**—**5**[2,3,9a,9b] and *p*-nitrophenyl 2-pyridinecarboxylate (**PNPP**)[13] were prepared by methods described in the literature.

cmc Values. Solutions of a surfactant of varied concentration were prepared with an appropriate aq solvent, and their surface tensions (γ/mN·m^{-1}) were measured by using Shimadzu Surface Tensometer ST-1 (Wilhelmy method). The cmc value was obtained as usual, being the concentration of the break point of a linear plot of γ vs. log C. The results are shown in Table 1.

Kinetics. The kinetics were carried out according to essentially the same method as reported previously.[2,3e,3f] A Hitachi-Horiba pH meter (F-8) was used for the pH determination and control. 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 10 μl of the **PNPP** stock solution into 3 ml of a buffer solution containing the desired reagents. Kinetic runs for fast rates were conducted

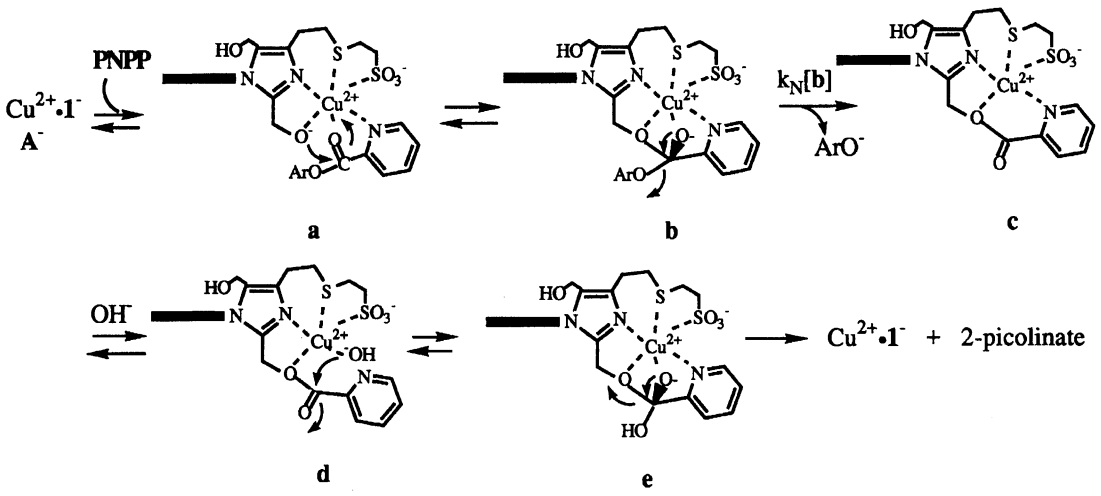


Fig. 9. Proposed acylation/deacylation steps in the surfactant Cu^{2+}•1^- complex-catalyzed hydrolysis of **PNPP**.

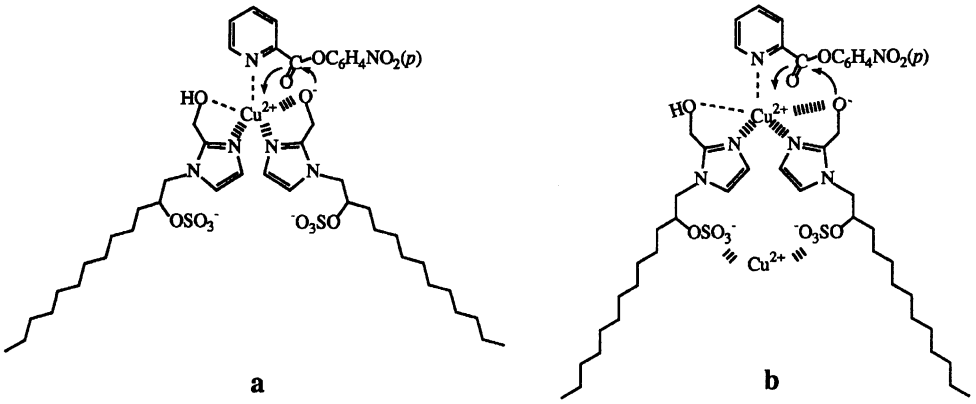


Fig. 10. Schematic illustration of 2 : 1 (**a**) and 1 : 1 (**b**) complexes between **2** and Cu^{2+}: a salt bridge with a second Cu^{2+} makes **b** less active than **a**, presumably due to an indirect steric effect.

by using a 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 (10 mL) 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—10) or at 320 nm (pH 4.5—6.0). The kinetic runs used for calculating the rate constants obeyed pseudo-first-order kinetics for at least 3 half-lives. These pseudo-first-order rate constants (k_{obsd}) were obtained by using $k_{obsd}=(2.30/t)\log[(OD_\infty-OD_0)/(OD_\infty-OD_t)]$.

Isolation of the 2-Pyridinecarboxylate Intermediate from the Reaction Mixture of 1 with PNPP by Quenching. In 5 ml of distilled water was dissolved 100 mg of **1** and 50 μl of aq $Cu(NO_3)_2$ (1 mol dm^{-3}); the solution was adjusted to pH 7 by aq NaOH. To this solution was added **PNPP** in 5 ml of chloroform under stirring; after 5 s the reaction mixture was quenched by adding a chelate-resin (Muromakku A-1, Muromachi Kagaku) 2 g under stirring to remove Cu^{2+}. When the reaction was further continued for more than one minute, it was over as the hydrolysis. The chelate-resin was removed by filtration and the filtrate was concentrated. The residue was treated with methanol/water (1 : 1) in order to recover unreacted **PNPP** (55 mg) as the precipitate. The filtrate was concentrated and the residue was chromatographed on a ODS column to give a mixture of the desired 2-pyridinecarboxylate and unreacted **1** (25 mg). The 2-pyridinecarboxylate was confirmed to take place predominantly on the 2-hydroxymethyl group by ^{1}H NMR analysis, as described in the text.

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.

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