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Hydrolytic Metalloenzyme Models. Metal Ion Dependent Site-selective Acylations of Hydroxyl Groups of Bis-imidazole Ligands Promoted by Zn^{2+} and Cu^{2+} Ions in Cationic Micelles

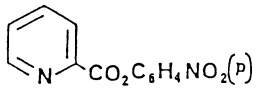
Kenji OGINO, Nobuyuki KASHIHARA, Tsunehisa FUJITA, Toshitomo UEDA,
Toshihide ISAKA, and Waichiro TAGAKI*
Department of Applied Chemistry, Faculty of Engineering,
Osaka City University, Sugimoto-3, Sumiyoshi-ku, Osaka 558

Co-micelles of cetyltrimethylammonium bromide and lipophilic bis-imidazole ligands having primary and secondary hydroxyl groups in their side chains were investigated for their catalytic activities in the hydrolysis of p-nitrophenyl picolinate in the presence of Zn^{2+} and Cu^{2+} at 25 °C. The kinetic and product analyses strongly suggested that the site-selective acylation of hydroxyl groups of ligands occurs via the formation of a tetrahedral for Zn^{2+} ion and a square planar complex for Cu^{2+} ion.

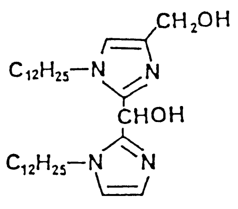
The catalytic activities of metalloenzymes are known to be dependent on metal ion species. The geometry of metal ion coordination is considered to be one important factor for the generation of full catalytic activities.1) There have been extensive studies on the divalent metal ion catalyzed ester hydrolysis as the models of hydrolytic metalloenzymes.2-5) Functionalyzed imidazoles6) and pyridines 7,8) comicelled with surfactants have also been studied as the metalloenzyme models. However, the informations have been very limited as for the active geometries of metal ion coordination in such model studies.

Previously,6) we reported that the metal ion complexes of N-dodecyl-2-hydroxymethylimidazole-Cu^{2+} and bis(N-octyl-2-imidazolyl)carbinol-Zn^{2+} are remarkably active catalysts in the hydrolysis of p-nitrophenyl picolinate(PNPP) when used in the micelles of cetyltrimethylammonium bromide(CTABr). It was also reported that the activation of the former monoimidazole ligand by Zn^{2+} ion and of the latter bis-imidazole ligand by Cu^{2+} ion is surprisingly small. These observations suggested that Zn^{2+} and Cu^{2+} ions activate the ligand hydroxyl groups by different modes of complexation.

In order to obtain more definite infromations on the difference between Zn^{2+} and Cu^{2+} ions in the activation of ligands, we have now prepared new lipophilic bis-imidazole ligands: (N-dodecyl-4-hydroxymethyl-2-imidazolyl)-(N'-dodecyl-2'-imidazolyl)carbinol 1 having both primary and secondary hydroxyl groups, and its methyl ether and tetrahydropyranyl ether derivatives, 2 and 3.9) These ligands are possible to take two different forms: a tetrahedral and a square planar forms in complexation with a metal ion as suggested by CPK molecular models. Interesting possibilities are that the former tetrahedral complexation would activate the secondary hydroxyl group and the latter square planar one would activate the primary hydroxyl group in acylation.



PNPP



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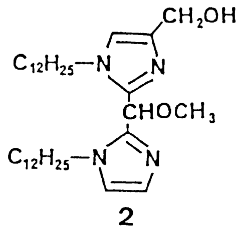

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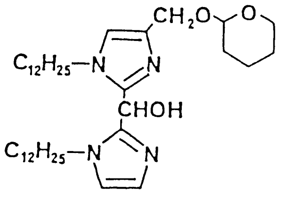

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In Figs. 1 and 2 are shown the plots of the observed pseudo-first-order rate constants(k_{obsd}) for the release of p-nitrophenol from PNPP as the function of Zn^{2+} or Cu^{2+} ion concentration. These figures indicate that, in the presence of fixed concentration of ligand, the rates tend to be saturated with increasing concentration of metal ion. As reported previously for the related systems, each saturation curve in Fig. 1 can be accounted for by assuming a reaction scheme: the formation of 1:1 complex of ligand-Zn^{2+} ion followed by the reaction between the complex and the substrate in a second-order manner. In the case of Fig. 2, the two saturation curves are complicating at first glance, but they can be accounted for by assuming the formation of an inactive 2:1 complex of ligand-Cu^{2+} ion at low metal ion concentration and its transformation into an active 1:1 complex as the metal ion concentration being increased.

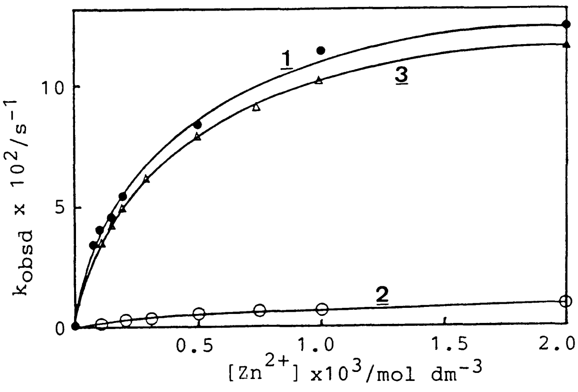


Fig. 1. Plots of $k_{obsd}(s^{-1})$ vs. Zn^{2+} concentration for p-nitrophenol release from PNPP in CTABr micelle at 25 °C, pH 7.03, [CTABr] $=1x10^{-2}$ mol dm^{-3}, [ligand]$=5x10^{-5}$ mol dm^{-3}, and [PNPP]$=1x10^{-5}$ mol dm^{-3}. The numbers in the figure are those of ligands.

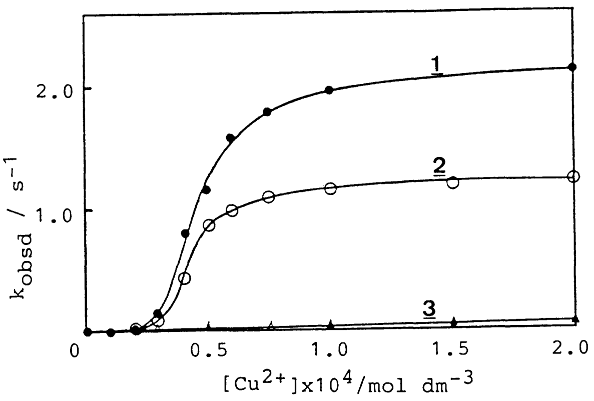


Fig. 2. Plots of $k_{obsd}(s^{-1})$ vs. Cu^{2+} concentration for p-nitrophenol release from PNPP in CTABr micelle at 25 °C, pH 7.03, [CTABr]$=1x10^{-2}$ mol dm^{-3}, [ligand]$=5x10^{-5}$ mol dm^{-3} and [PNPP]$=1x10^{-5}$ mol dm^{-3}. The numbers in the figure are those of ligands.

It is important to notice that the ligand 1 is activated by both Zn^{2+} and Cu^{2+} ions, in sharp contrast to the cases of the ligands 2 and 3. Namely, the ligand 3 is activated by Zn^{2+} ion, equally well as in the case of 1(Fig. 1), but not by Cu^{2+} ion(Fig. 2). In a reversed manner, the ligand 2 is activated by Cu^{2+} ion(Fig. 2), but not by Zn^{2+} ion(Fig. 1). These results clearly indicate that Zn^{2+} ion activates the secondary hydroxyl groups while Cu^{2+} ion activates the primary ones. An almighty activity of 1 is the result of selective activation of primary and secondary hydroxyl groups by Cu^{2+} and Zn^{2+} ions, respectively. These results also indicate that the free hydroxyl groups are essential for the generation of catalytic activity.

The above selectivity demonstrated by kinetic means was further confirmed by product analysis. A typical product analysis by HPLC is shown in Fig. 3 which was obtained by analyzing a reaction mixture of PNPP(2×10^{-4} mol dm^{-3}), 1(2×10^{-4} mol dm^{-3}), Zn^{2+} or Cu^{2+}(5×10^{-4} mol dm^{-3}) and CTABr(1×10^{-2} mol dm^{-3}), after one minute reaction(pH 7, 25 °C).[10] The figure indicates two peaks A and B and they were identified to be the peaks of the acylated intermediates, 4 and 5, respectively.[11] The relative peak area of A and B was observed to depend on the reaction time.[12] However, at the initial stage of reaction, the predominant peak was A in the case of Zn^{2+} ion, while that was B in the case of Cu^{2+} ion. Thus it is quite clear that the initial acylation takes place selectively on the secondary hydroxyl group of 1 to form 4(peak A) in the presence of Zn^{2+} ion, while it takes place selectively on the primary hydroxyl group to form 5(peak B) in the presence of Cu^{2+} ion.

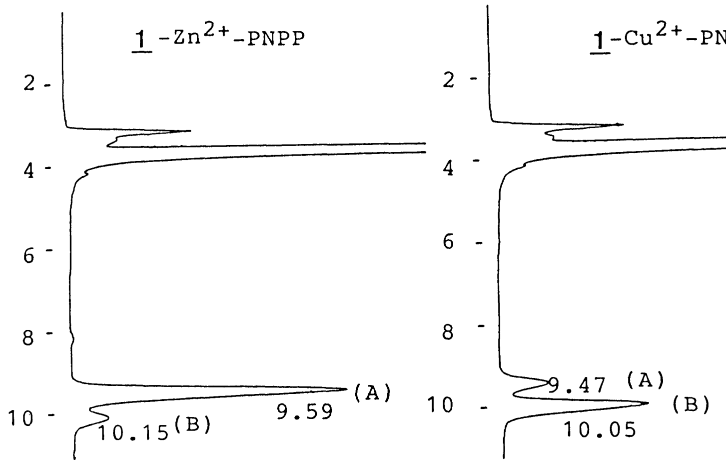
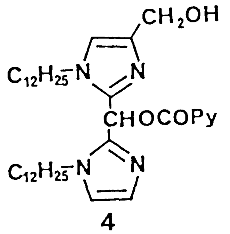


Fig. 3. HPLC analysis of acylated ligands.



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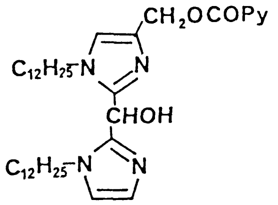

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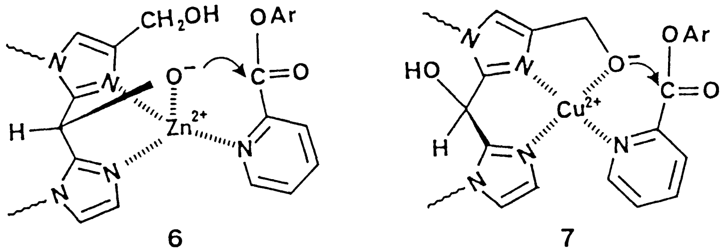
As illustrated in Fig. 4, the above metal ion dependent site-selective acylation of hydroxyl groups seems to be nicely explained by assuming a tetrahedral geometry for the ternary complex of 1-Zn^{2+}-substrate 6 and a square planar one for the complex of 1-Cu^{2+}-substrate 7 at the transition states or in the reactive intermediates. In each complex, a metal ion activates the hydroxyl group by tight coordination. The tight coordination of ligand hydroxyl group to metal ion



6 7

Fig. 4. Schematic illustration of the ternary complexes.

would lower the pK_a of hydroxyl group. From the pH-rate profiles by using the rate constants at the saturation levels(Figs. 1 and 2) and pH region 4.5-8.5, the pK_a's of 1-Cu^{2+}, 2-Cu^{2+}, and 1-Zn^{2+} complexes were determined kinetically to be 6.1, 6.3, and 7.5(25 °C), respectively. Thus the hydroxyl groups of these bis-imidazole ligands are fully activated for acylation under the neutral conditions, by coordination to Cu^{2+} or Zn^{2+} ion.

Finally, it should be mentioned that the present micellar reactions are catalytic ones. Namely, the hydrolysis of PNPP proceeds to the completion under the conditions of an excess of substrate over the ligands in both cases of Cu^{2+} and Zn^{2+} ions which means that the deacylation of acylated intermediates is also fast. Further works are in progress to prepare surfactant bis-imidazole ligands.

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References

1) "The Enzymes," 3rd, ed by P. D. Boyer, Academic Press, New York(1971), Vols. III and IV.
2) R. J. P. Williams, Pure Appl. Chem., 54, 1889(1982).
3) R. S. Brown, D. Salmon, N. J. Curtis, and S. Kusama, J. Am. Chem. Soc., 104, 3188(1983).
4) T. H. Fife and T. J. Przystas, J. Am. Chem. Soc., 108, 4631(1986).
5) K. Ogino, K. Shindo, T. Minami, and W. Tagaki, Bull. Chem. Soc. Jpn., 56, 1101, (1983).
6) T. Eiki, S. Kawada, K. Matsushima, M. Mori, and W. Tagaki, Chem. Lett., 1980, 997; K. Ogino, I. Tomita, T. Machiya, and W. Tagaki, ibid., 1982, 1875; W. Tagaki and K. Ogino, Top. Curr. Chem., 128, 143(1985).
7) R. Fornasier, D. Milani, P. Scrimin, and U. Tonellato, J. Chem. Soc., Perkin Trans. 2, 1986, 233.
8) S. H. Gellman, R. Petter, and R. Breslow, J. Am. Chem. Soc., 108, 2388(1986).
9) Lithiated N-dodecyl-4-tetrahydropyranyloxymethylimidazole was reacted with N-dodecylimidazole-2-carboxaldehyde to give 3. 1 was obtained by removal of tetrahydropyranyl protecting group of 3. 2 was obtained by methylation of 3 followed by removal of tetrahydropyranyl group. The structures and purities of these ligands were checked by ^1H and ^{13}C-NMR spectra and HPLC analysis.
10) The reaction mixture was quenched with EDTA solution and the chloroform extract was subjected to HPLC analysis.
11) In the preparative scale experiments, these 4 and 5 were obtained as the major products when the reactions were conducted in the presence of Zn^{2+} and Cu^{2+} ions, respectively. Their structures were determined based on ^{13}C-NMR analyses: the ^{13}C signals of the hydroxylated carbons shifted significantly on acylation in both cases of primary and secondary hydroxyl groups.
12) This was partly due to the different rates of deacylation of 4 and 5 depending on the metal ion species.

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