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Hydrolysis of p-Nitrophenyl Picolinate by N-Aminimides Having Metal Binding Site and Hydroxyl Group in the Presence of Zinc(II) Ion

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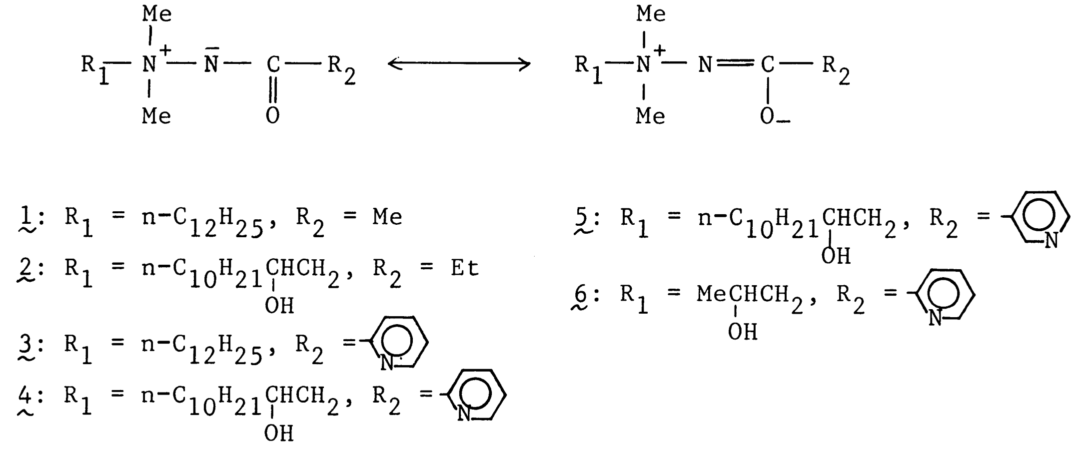
It was found that N-aminimides possessing a metal binding site and a hydroxyl group facilitate transesterification of p-nitrophenyl picolinate(PNPP) to the hydroxyl group in the presence of Zn^{2+} in aqueous solution.

We have shown that hydrophobic N-aminimides behave as non-ionic surfactants in aqueous solution.[1)] The zwitter ionic N-aminimides, however, are considered to interact with both metal cation and its counter anion. In fact, a hydrophobic N-aminimide is known to serve as a carrier for transport of alkali metal cation through a liquid membrane,[2)] and also as a phase-transfer catalyst for Finkelstein reactions.[1)] In this sense, the zwitter ionic N-aminimide could be regarded as a unique molecule.

Meanwhile Tagaki et al. have shown models for hydrolytic metalloenzymes such as carboxypeptidase A and alkaline phosphatase by employing hydrolysis of PNPP with imidazole and pyridine derivatives having hydroxyl groups in the presence of divalent metal cations under non- and micellar conditions.[3)] Their micellar systems are co-micelles of the lipophilic ligands and CTABr. These prompted us to study the hydrolytic activity of a micelle-forming N-aminimide itself having a metal binding site and a hydroxyl group in the presence of Zn^{2+}(Scheme 1).



Scheme 1.

In the case that R_2 is 2-pyridyl groups (3 and 4), Zn^{2+} would be bound at the pyridyl nitrogen and the oxyanion in a similar manner to that of 2-pyridine carbaldehyde and Zn^{2+}.[4] Such metal chelation would neutralize the negative charge in the N-aminimide to provide a cationic N-aminimide involving Zn^{2+} near the ammonium cation. In other words, zwitter ionic micelles of the N-aminimides are converted to the cationic micelles in the presence of Zn^{2+}, in which the hydroxyl group would be activated by the cationic micelles[5] or/and by co-ordination to the chelated Zn^{2+}.[6]

Interaction of 4 and Zn^{2+} was briefly examined spectrophotometrically in aqueous solution (pH 7.0, 0.05 M lutidine buffer). Absorption spectrum of 4 (6.0×10^{-4}M in 1 mm cell)[λ_{max} 263 nm (ε, 8500)] was found to shift to λ_{max} 270 nm (ε, 12000) with the increase of $[Zn^{2+}]$($0 - 1.2 \times 10^{-3}$M), whereas such a spectral shift was not observed for both 2 and 5, suggesting that the chelation of Zn^{2+} occurs at the 2-pyridyl nitrogen and the oxyanion.

Pseudo first-order rate constants (k_{obsd}) for hydrolysis of PNPP were determined as usual by monitoring the release of p-nitrophenol at 400 nm in the conditions of excess ligands over PNPP in aqueous solution. In the absence of Zn^{2+}, the rates were little affected by the N-aminimides (1-5) due to the non-ionic character of these surfactants.[1] In the presence of Zn^{2+}, however, relatively large accelerations were observed only for the N-aminimides having both 2-pyridyl and hydroxyl groups (4 and 6). As shown in Fig. 1, the rates for 4 and 6 increase by increasing [ligand] to reach maximum rates at [4] : $[Zn^{2+}]$ = 2 : 1 (25-fold), and at 1 : 1 complex for 6 (14-fold). The N-aminimides having a hydroxyl group alone (2) and 3-pyridyl and hydroxyl groups (5) exhibit no rate enhancements, although 3 shows a small rate acceleration (5.5-fold).[7] These observations suggest that the reactive species are the 2 : 1 complex of 4 and Zn^{2+} in micellar and the 1 : 1 complex of 6 and Zn^{2+} in non-micellar conditions,[8] although it has not been clear why the 2 : 1 complex is more favourable in micellar systems. Formation of the 2 : 1 complex was also confirmed by isolation as follows. Namely, addition of $Zn(NO_3)_2 \cdot 6H_2O$ (360 mg, 1.2 mmol) in H_2O (100 ml) to a stirred solution of 4 (840 mg, 2.4 mmol) in H_2O (100 ml) gave white precipitates immediately, which were collected by filteration, washed well with water, and dried over a vaccum desiccator (970 mg, 91%). Elemental analysis showed formation of the 2 : 1 complex (Found: C; 54.14, H; 7.98, N; 12.15%. Calcd for $2(C_{20}H_{33}N_3O_2)\text{-}Zn(NO_3)_2$: C; 54.05, H; 7.94, N; 12.62%).

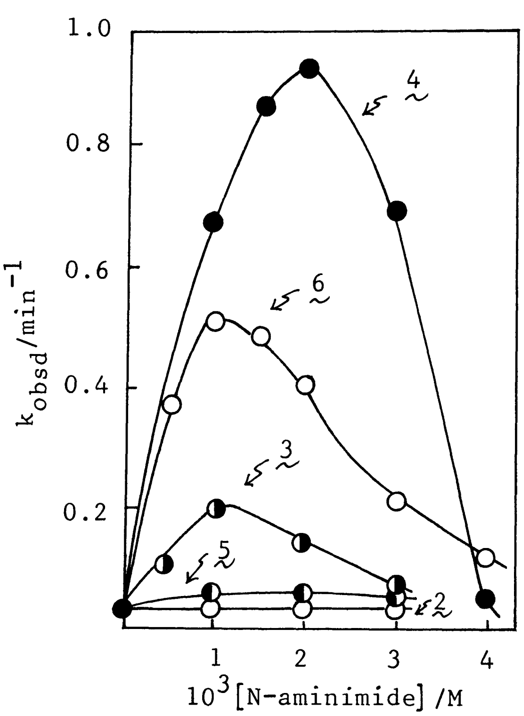


Fig. 1. Plots of k_{obsd} vs. [N-aminimide] in the presence of Zn^{2+}. [PNPP] = 5.0×10^{-5}M, $[Zn^{2+}]$ = 1.00×10^{-3}M, pH 7.41, 25 °C.

The effect of Zn^{2+} concentration was

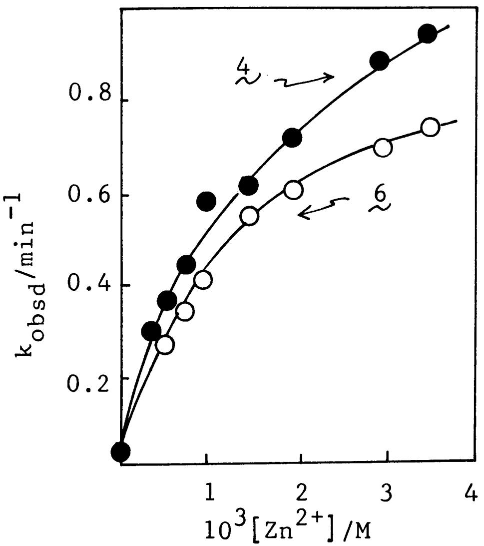


Fig. 2. Plots of k_{obsd} vs. $[Zn^{2+}]$. [PNPP] = 5.0×10^{-5} M, [**4**] = [**6**] = 1.0×10^{-3} M, pH 7.41, 25 °C.

examined by employing **4** and **6** (Fig. 2).[9] The saturation curves were analyzed according to Tagaki's kinetic treatments.[3,10] The results together with typical kinetic data are summarized in Table 1. As can be seen in Table 1, the rate accelerations due to Zn^{2+}-**4** and Zn^{2+}-**6** are much larger than those due to Zn^{2+} or **4** alone, indicating that Zn^{2+} and **4** or **6** function co-operatively. Similar results have been shown by Tagaki et al. by employing N-alkyl-2-hydroxymethylimidazole derivatives and Zn^{2+}.[3)] Namely, the 2 : 1 complex is a reactive species for the N-dodecyl derivative solubilized in CTABr with $K = 1.70 \times 10^5\ M^{-2}$ and $k_c = 22.4 \times 10^4\ M^{-1}min^{-1}$ at pH 7.0, whereas the 1 : 1 complex for the N-methyl one with $K = 1.04 \times 10^2\ M^{-1}$ and $k_c = 8.16 \times 10^3\ M^{-1}min^{-1}$. It should be noted that the ratios of the k_c values between micellar and non-micellar systems are 27 for the imidazole derivatives, and 4.5 for the N-aminimides (Zn^{2+}-**4** vs. Zn^{2+}-**6**).

Table 1. The rate constants for hydrolysis of PNPP[a)]

Catalyst	k_{obsd}/min^{-1}	Rel. rate	K/M^{-1} or M^{-2}	$k_c/M^{-1}min^{-1}$
None	1.67×10^{-3}	1.0	—	—
4	1.70×10^{-3}	1.0	—	—
Zn^{2+}	4.49×10^{-2}	27	—	—
Zn^{2+}-**4**[b)]	9.23×10^{-1}	550	$1.6 \times 10^5\ M^{-2}$	5.8×10^3
Zn^{2+}-**4**-CTABr[b,c)]	1.54	920	—	—
Zn^{2+}-**4**-$C_{12}E_6$[b,d)]	1.62	970	—	—
Zn^{2+}-**6**	5.10×10^{-1}	310	$7.3 \times 10^2\ M^{-1}$	1.3×10^3

a) [PNPP] = 5.0×10^{-5} M, [N-aminimide] = $[Zn(NO_3)_2 6H_2O]$ = 1.00×10^{-3} M, pH 7.41 (0.05 M lutidine buffer), 25 °C. b) [**4**] = 2.00×10^{-3} M. c) [CTABr] = 4.0×10^{-3} M. d) $n\text{-}C_{12}H_{25}(OC_2H_4)_6OH$, $[C_{12}E_6]$ = 4.0×10^{-3} M.

To achieve large rate enhancements, the Zn^{2+}-ligands complex must form a complex with PNPP, in which the hydroxyl group of the ligands must be favourably oriented for intracomplex nucleophilic attack on the carbonyl group of PNPP. Thus, the small rate enhancement due to micellization (Zn^{2+}-**4** vs. Zn^{2+}-**6**) suggests that the orientation of the hydroxyl group and >C=O of PNPP in the complex is less favourable for the 2 : 1 complex than the 1 : 1 complex compared with the imidazole derivatives.

No difference between Zn^{2+}-**4**-CTABr and Zn^{2+}-**4**-$C_{12}E_6$ suggests that the reaction environment provided by Zn^{2+}-**4** is not affected by the additional surfactants.[11)]

Under the conditions of excess PNPP over **4** in the presence of Zn^{2+}, burst kinetics of initially fast and subsequently slow release of p-nitrophenol were observed, indicating that transesterification occurs to the hydroxyl group co-ordinated to the Zn^{2+} in the complex.[12)]

The present study demonstrates that N-aminimides having a metal binding site and a hydroxyl group can be used as the models for the hydrolytic metalloenzymes in the presence of Zn^{2+} in aqueous solution if the co-ordination of the hydroxyl group to the chelated Zn^{2+} is possible. A next problem is how to accelerate the deacylation step to obtain a turnover catalyst.

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References

1) S. Inokuma, E. Kameyama, and T. Kuwamura, Yukagaku, 32, 332 (1983).

2) S. Tsuchiya, Y. Yamada, and M. Seno, J. Chem. Soc., Chem. Commun., 1983, 995.

3) T. Eiki, M. Mori, S. Kawada, K. Matsushima, and W. Tagaki, Chem. Lett., 1980, 1431; K. Ogino, K. Shindo, T. Minami, W. Tagaki, and T. Eiki, Bull. Chem. Soc. Jpn., 56, 1101 (1983); W. Tagaki and K. Ogino, "Topics in Current Chemistry," ed by E. L. Bochke, Springer-Verlag, Tokyo (1985), p. 143, and references are cited therein.

4) W. Hughes, R. H. Prince, and P. Wyeth, J. Inorg. Nucl. Chem., 40, 713 (1978).

5) C. A. Bunton and L. C. Inoescu, J. Am. Chem. Soc., 95, 2912 (1973); C. A. Bunton and S. Diaz, J. Org. Chem., 41, 33 (1976); M. A. Moss, R. C. Nahas, S. Ramaswami, and W. J. Sadero, Tetrahedron Lett., 1975, 3379; Y. Yano, Y. Yoshida, Y. Tamura, and W. Tagaki, J. Chem. Soc., Perkin Trans. 2, 1979, 1128.

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

7) This involves the effect of CTABr (1.0×10^{-3}M), which was added to dissolve 3 (1.0×10^{-3}M).

8) The cmc's were determined by the method of surface tension measurements; 4.0×10^{-4}M for 4 and 8.0×10^{-5}M for Zn^{2+}-4.

9) The rate at [Zn^{2+}] = 4.0×10^{-3}M could not be determined due to precipitation.

10) K and k_c are the association constants of Zn^{2+} and the ligands, and the second-order rate constants for p-nitrophenol release from PNPP by the Zn^{2+}-ligands.[3)]

11) The concentration effects of CTABr and $C_{12}E_6$ were examined for the Zn^{2+}-4 system under the conditions of the maximum rates as shown in Fig. 1, exhibiting the same effects.

12) The pH - log k_{obsd} plots for Zn^{2+}-4 showed a straight line unity slope up to pH 8.5, indicating that pK_a of the hydroxyl group is larger than 8.5. Above pH 8.5, the rates could not be determined due to precipitation.

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