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A MICELLAR MODEL OF ZINC CONTAINING METALLOENZYMES

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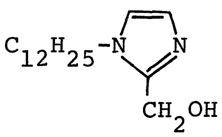
N-Dodecyl-2-hydroxymethylimidazole (**1**) was found to be unusually active in the transesterification toward p-nitrophenyl picolinate (**4**), when the reaction was carried out in the presence of CTABr micelle and zinc ion.

Although micellar catalysis as a model of enzymic catalysis has been extensively studied for the past decade, a very few examples has been known which mimic hydrolytic metalloenzymes.[1,2] The role of zinc ion at the active site of zinc containing metalloenzymes has also been the subject of intensive studies in recent years.[3] The zinc ion may function to activate water in carbonic anhydrase[4] or to activate serine hydroxyl group in alkaline phosphatase.[5] In the former enzyme, the imidazole group of histidyl residue is known to act as the ligand of zinc ion. Therefore it is important to device a model system in which imidazole and hydroxyl groups act cooperatively in the presence of zinc ion for better understanding of the mechanism of catalyses of such metalloenzymes. We now wish to report that N-dodecyl-2-hydroxymethylimidazole (**1**) is unusually active when incorporated in a cationic micelle and used in the presence of zinc ion toward the transesterification of p-nitrophenyl picolinate (**4**).

The reaction was followed by observing the release of p-nitrophenol from **4** spectrophotometrically.[6,7] The rate was first-order with respect to the substrate (**4**) under the conditions of excess ligand (**1**) over the substrate. However, under the reversed conditions of the excess substrate over the ligand, typical burst kinetics were observed with initially rapid, but later slower release of p-nitrophenol. Such burst kinetics indicate that the transesterification to give acylation of 2-hydroxyl group of **1** is rapid, but the subsequent deacylation to regenerate the ligand is slow.[8] Thus, in order to examine the transesterification step, the following kinetics were carried out under the conditions of the excess ligand over the substrate.



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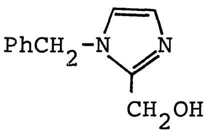

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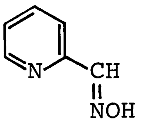

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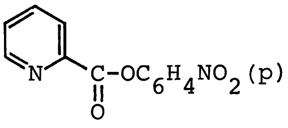

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The pseudo-first-order rate constants(k_{obs}) for the release of p-nitrophenol increased by increasing zinc ion concentration in a fashion to give saturation kinetics as shown in Fig. 1.

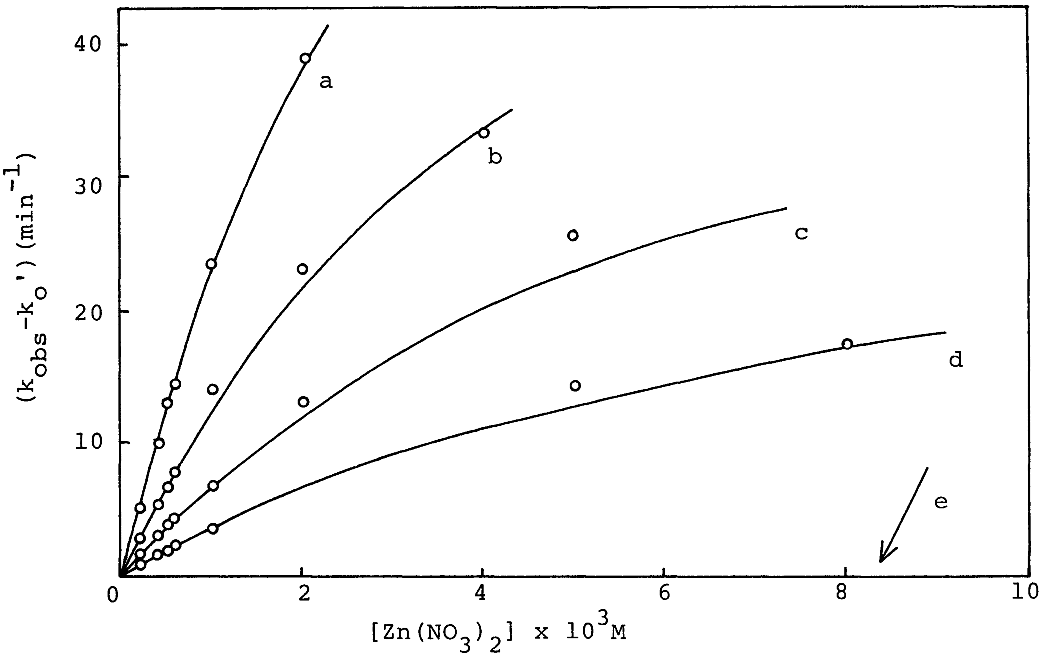


Fig. 1. Plots of pseudo-first-order rate constants for the release of p-nitrophenol from **4** as a function of zinc ion concentration: ligand (**1**) concentrations($L_T \times 10^4$M) are (a) 10, (b) 7, (c) 5, (d) 3.5, and (e) 0; solid lines are calculated ones based on the constants in Table 1; see Table 1 for the other reaction conditions.

The saturation curves in the figure can be nicely analyzed by assuming a 2:1 complex of **1** (L) and zinc ion (M) according to a reaction scheme of eqs 1-3. The scheme allows to derive the rate eqs 4-6,[9] and the slopes and intercepts of plots of eq 6 allow to calculate the k_c and K values as listed in Table 1. The table also includes the data of other related non-micellar systems. Here it should be noted that there is a difference in forming a zinc ion complex between micellar and non-micellar ligands. As reported previously the 1:1 complex fits the rate data in the cases of non-micellar ligands.[7,10] Why such difference occurs is not clear at present, although it is conceivable that a 2:1 complex is more stable in a micellar phase. Nevertheless the k_c values may be compared as a measure of activities of these ligand-zinc ion complexes. It is interesting to note that the complex of **1** is 100 times more active than that of **3**, which is

$$2L + M \overset{K}{\rightleftharpoons} \text{complex} \qquad (1)$$

$$S + \text{complex} \xrightarrow{k_c} \text{products} \qquad (2)$$

$$S \xrightarrow{k'_o} \text{products} \qquad (3)$$

$$k'_o = k_o + k_L L_T + k_M M_T \qquad (4)$$

$$k_{obs} = k'_o + \frac{k_c K L_T^2 M_T}{1 + K L_T^2 + 4 K L_T M_T} \qquad (5)$$

$$\frac{1}{(k_{obs}-k'_o)} = \frac{4}{k_c L_T} + \frac{1 + K L_T^2}{k_c K L_T^2} \cdot \frac{1}{M_T} \qquad (6)$$

Table 1. Rate Constants for p-Nitrophenol Release from p-Nitrophenyl Picolinate (4) in the Presence of Zn (II) Ion and Ligand a)

Ligand	pH	K	rate constant
None	b	-	$k_{obs} = 7.90 \times 10^{-4} min^{-1}$
CTABr d)	b	-	$k_{obs} = 2.60 \times 10^{-3} min^{-1}$
Zn(II)	b	-	$k_M = 15.3\ M^{-1} min^{-1}$
Zn(II) + CTABr d)	b	-	$k_M = 16.7\ M^{-1} min^{-1}$
1 + CTABr d)	b	-	$k_L = 4.32 \times 10^{2} M^{-1} min^{-1}$
1 + Zn(II) + CTABr d)	b	$7.45 \times 10^{4} M^{-2}$	$k_c = 4.30 \times 10^{5} M^{-1} min^{-1}$ e)
2	c	$162\ M^{-1}$	$k_c = 1.00 \times 10^{4} M^{-1} min^{-1}$ f)
3	c	$557\ M^{-1}$	$k_c = 4.00 \times 10^{3} M^{-1} min^{-1}$ f)

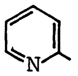
a) The reaction was carried out in 2,6-lutidine-HNO_3 buffer (0.05 M), μ=0.1(KNO_3), 25°C; The concentrations were varied: $Zn(NO_3)_2$=0-10^{-2}M, ligand=0-1x10^{-3}M; The concentrations of the substrate (4) (5x10^{-5}M) and CTABr (5x10^{-3}M) were kept constant.

b) 7.20 c) 7.06 d) Contains 1% ethanol (volume)

e) Based on the 2:1 complex of ligand and Zn(II) ion (see text)

f) Based on the 1:1 complex of ligand and Zn(II) ion (ref. 7,10)

Table 2. Pseudo-first-order Rate Constants in the Transesterification between 1 and p-Nitrophenyl Carboxylates ($RCO_2C_6H_4NO_2$) a)

R	k'_c/k_o
CH_3	29
C_2H_5	82
	30000

a) Catalyst system: Ligand (1)=Zn(II)=1x10^{-3}M and CTABr=5x10^{-3}M. The medium: pH 7.06 (0.05 M, 2,6-lutidine-HNO_3 buffer), μ=0.1 (KNO_3), 25°C. Substrate=5x10^{-5}M.

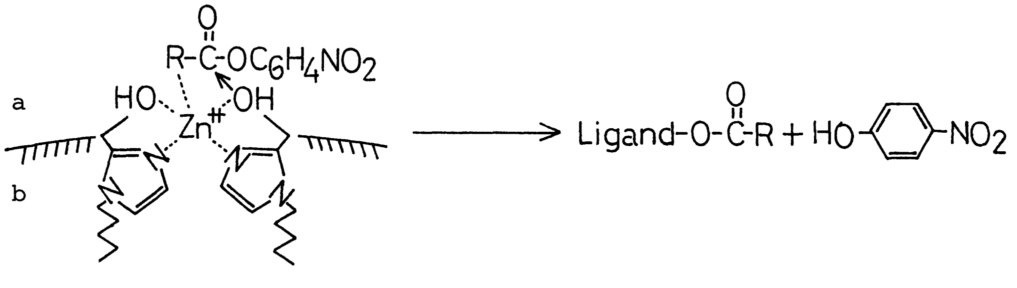


Fig. 2. A schematic illustration of transesterification between the 2:1 complex of 1 and zinc ion and p-nitrophenyl carboxylates in the CTABr micelle: (a) aqueous phase, (b) micellar phase.

well known as an effective catalyst (or reagent) for the reactions of some carboxylic acid esters[11)] and phosphates.[12,13)] Another aspect of reactivity is the substrate specificity.

As shown in Table 2, the rate in the absence of complex (k_o) is enhanced 30,000 fold in the presence of 10^{-3}M each of 1 and zinc ion (k'_c). Rate enhancement is also observed for other substrates, but its extent is relatively small. Presumably, as illustrated in Fig. 2, positioning of substrate at proper orientation for reaction to occur by chelation with metal ion is important in addition to the preequilibrium binding of substrate into micellar phase by apolar interaction. The micellar effects of CTABr are complex and will be the subject of further detailed studies. It is certain, however, that the presence of CTABr above CMC is essential for both solubilization of the system and the rate enhancement.

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References and Notes

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7) T. Eiki, S. Kawada, K. Matsushima, M. Mori, and W. Tagaki, Chemistry Letters, 1980, 997; Ligand 1 and 2 were readily prepared by the condensation of corresponding N-alkylimidazoles with formaldehyde.
8) The acylated intermediate, 2-picolinate of 1 was detected in the reaction mixture by high speed liquid chromatography.
9) K: association constant, k_c: catalytic rate constant, k'_o: combined rate constant for other than that of complex, and L_T and M_T: total concentrations of ligand and zinc ion.
10) $k_{obs}=k'_o + k_c K L_T M_T / (1 + KL_T + KM_T)$
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