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ENANTIOSELECTIVE ESTER HYDROLYSIS CATALYZED BY A MICELLAR MODEL OF ZINC ENZYMES

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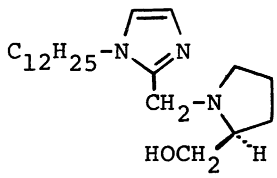
N-Laurylimidazoles containing L-2-pyrrolidinemethanol function gave large rate enhancements[$k^{a}_{obsd}(L)/k^{o}_{obsd}(L)$=56-401] and reasonable enantioselectivities[$k^{a}_{obsd}(L)/k^{a}_{obsd}(D)$=1.40-3.92] in the presence of zinc ion and CTABr micelle for the hydrolysis of p-nitrophenyl L- and D-N-benzyloxycarbonylphenylalanates.

Model studies for the stereoselective hydrolysis of enantiomeric esters have been the subject of continued interests in order to understand the mechanism of proteolytic enzyme catalyses,[1)] and some of micellar systems involving chiral histidine-functionalized surfactants have been found to exibit moderate or high enantioselectivities.[2)] However, there has been no report for the micellar catalysis dealing with the metal ion catalyzed acyl-transfer reactions involving coordinated lipophilic ligands with chiral centers. Metal ion catalyzed reactions themselves have been extensively investigated in recent years as the model reactions of metalloenzymes, such as carboxypeptidase A, carbonic anhydrase, or related enzymes.[3)] We previously reported that N-substituted (2-hydroxymethyl)imidazole-metal ion complexes presented the high catalytic activity for the hydrolysis of p-nitrophenyl picolinate as a simple and good model of the hydrolytic metalloenzymes.[4)]

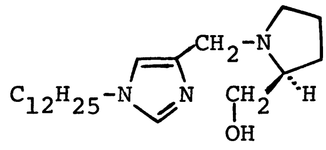
In the present paper, we wish to report the stereoselective hydrolysis of L- and D- N -benzyloxycarbonylphenylalanine p-nitrophenyl esters[L- and D-(Z)-Phe-PNP: L-1 and D-1][5)] catalyzed by N-laurylimidazolyl derivatives having L-2-pyrrolidine-methanol moiety(L-2, L-3, and LL-4)[5)] in the presence of zinc ion in CTABr micellar systems. The present ligands would be favorably coordinated with metal ion to activate the hydroxyl group of ligands for nucleophilic attack as suggested by the CPK model structures, and would also serve to bind a lipophilic substrate by hydrophobic interaction.

$PhCH_2OCONH\overset{*}{C}HCOOC_6H_4NO_2$(p)
$\qquad\qquad\quad |$
$\qquad\qquad CH_2Ph$

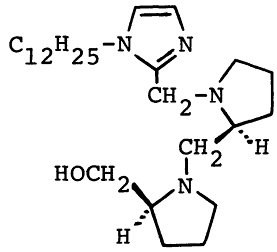
L-1 and D-1



L-2



L-3



LL-4

The rates of reaction were followed by observing the release of p-nitrophenolate from the substrate(L-1 or D-1) spectrophotometrically in 0.1 mol dm^{-3} of 2,6-lutidine buffer in CTABr micelle at pH 7,51 at 25 °C. In all cases, under the condition of excess ligand over the substrate, good pseudo-first-order relations(rate constants, k^a_{obsd}) were obtained up to completion of reaction. The kinetic data are summerized in Table 1.

Table 1. Pseudo-first-order rate constant($k^a_{obsd} \times 10^3$ sec^{-1}) for p-nitrophenol release from L- or D-(Z)-Phe-PNP in CTABr micelle.

conditions	k^a_{obsd}(L)	k^a_{obsd}(D)	$k^a_{obsd}(L)/k^o_{obsd}(L)$	$k^a_{obsd}(L)/k^a_{obsd}(D)$
none	0.0735	-	-	-
Zn^{2+}	0.0797	-	1.1	-
L-2	0.408	0.359	5.6	1.14
L-3	0.226	0.159	3.1	1.42
LL-4	0.503	0.209	6.8	2.41
L-2-Zn^{2+}	21.3	11.4	290	1.87
L-3-Zn^{2+}	4.14	2.95	56	1.40
LL-4-Zn^{2+}	29.5	7.53	401	3.92

observed at pH 7.51(2,6-lutidine 0.1 mol dm^{-3} buffer), μ=0.1(KNO_3), [ester]=1×10^{-4} mol dm^{-3}, [lig]=1×10^{-3} mol dm^{-3}, [Zn^{2+}]=2×10^{-3} mol dm^{-3}, [CTABr]=1×10^{-2} mol dm^{-3} at 25 °C.

Zinc ion itself showed essentially no catalytic activity, presumably because of the lack of coordination toward the substrate. In the absence of metal ion, the rate enhancements by the ligand alone were also relatively small, and their enantioselectivities, $k^a_{obsd}(L)/k^a_{obsd}(D)$, were comparable to those for histidine-functionalyzed surfactants.[2a-f)]

Large rate enhancements were observed only in the presence of both the ligand

and zinc ion, thus zinc ion was indicated to play an important role for the activation of ligands. The reactivity, $k^{a}_{obsd}(L)/k^{o}_{obsd}(L)$, toward L-1 is in the order of LL-4 > L-2 > L-3, and the same order is also observed in the enantioselectivity, $k^{a}_{obsd}(L)/k^{a}_{obsd}(D)$.

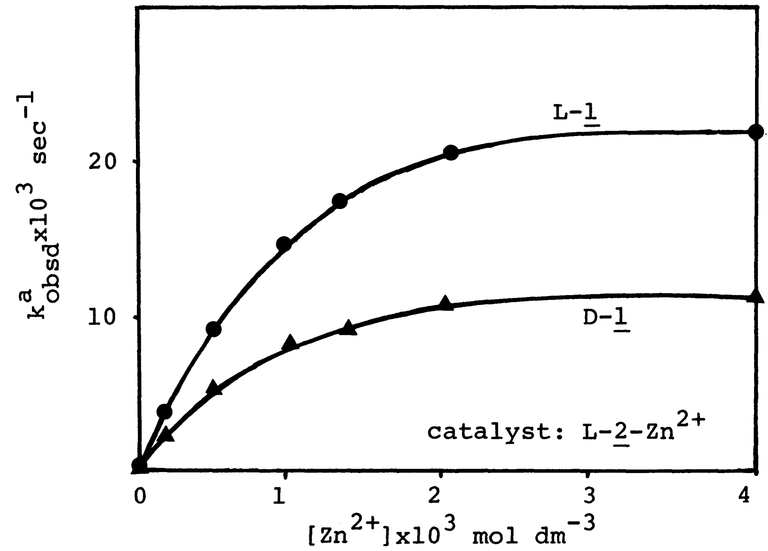


Figure 1. Plots of pseudo-first-order rate constants for the release of PNP from L-1 and D-1 as a function of zinc ion concentration. See Table 1 for other conditions.

The rate constants of L-2-Zn^{2+} system increased by increasing zinc ion concentration as shown in Figure 1. The saturation curves in the figure correspond to the formation of the 1:1 complex of zinc ion and ligand.[6] And its enantioselectivity (1.87) is rather larger than that of the ligand itself(1.14).

Under the condition of five molar excess of substrate over the ligand, typical burst kinetics were observed; initial fast release followed by slow release of p-nitrophenol as shown in Figure 2. Such a biphasic behavior is an evidence for a two step process involving the acylation of hydroxyl group of catalyst followed by the rate determining deacylation to regenerate the catalyst.[3a,c,d,4c] It is interesting to note that the second deacylation step also occurs enantioselectively.[7] Presumably it is due to the deacylation occurring by the attack of zinc ion-coordinated hydroxide ion which in principle, should be enantioselective as in the hydroxyl group of ligand.

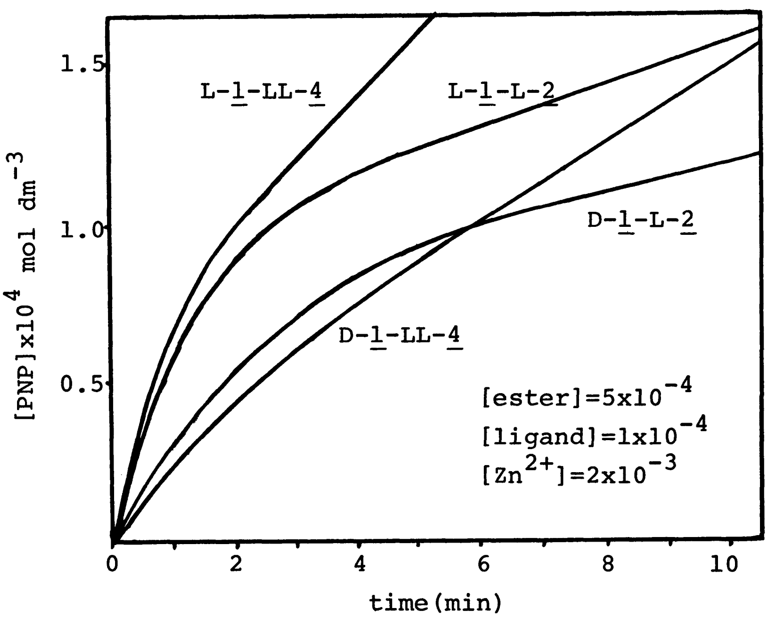


Figure 2. Burst kinetics: [ester]/[ligand]=5.0

Alternatively, the enantioselectivity is also expected when the free hydroxide ion attack the coordinated carbonyl group of acyl-intermediate with zinc ion. At any rate, the rates of both steps of acylation and deacylation for L-ester were observed to be larger than those for D-ester. The enantioselectivities of deacylation steps, $k^{d}_{obsd}(L)/k^{d}_{obsd}(D)$, were calculated from Figure 2 to be about 2.2 for L-2-Zn^{2+} and 1.8 for LL-4-Zn^{2+}, respectively, which are somewhat reversed magnitudes as compared to those for the acylation step (Table 1).

The above results have demonstrated for the first time that the stereoselectivity of proteolytic metalloenzymes can be modeled by micellar systems. We are currently preparing various substrates and ligands to extend the present results.

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5). L- and D-(Z)-Phe-PNP were prepared by DCC/$CHCl_3$ condensation of p-nitrophenol and (Z)-Phe. For the procedures, see M.Goodman and K.C.Stueben, J.Am.Chem.Soc., 81, 1959, 3980; ref. 2d. The ligands L-2, L-3, and LL-4 were synthesized by the reactions of chlorinated imidazolyl derivatives with commercial L-2-pyrrolidine-methanol.

6). The 1:1 complex of ligand and zinc ion should be active for acyl-transfer reactions. Details will appear in a full paper.

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