Catalytic Reaction of Model Zinc(II) Complex for Active Sites of Mono-nuclear Zinc-peptidases

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Catalytic hydrolysis reaction of amide by designed zinc-complex model for active sites of mono-nuclear zinc-peptidases has been investigated. The effect of carboxylate ligand of the model complex on hydrolysis activity was revealed by kinetic analyses.

Several zinc-peptidases, such as *carboxypeptidases*,[1] *thermolysin*,[2] *pseudolysin*,[3] *bacillolysin*,[4] *steptomyces*,[5] and *sonic hedgehog*,[6] have mono-nuclear zinc active site bound to two histidine imidazoles, one carboxylate of glutamic or aspartic acid, and one water molecule. In these enzymatic hydrolysis of peptide, nucleophile means a deprotonated zinc-bound water, Zn-OH.[7]

Many studies on the above mechanism have been reported using the model complexes for zinc-peptidases.[8-10] Also the another mechanism, the zinc ion acts as general acid and external carboxylate or hydroxide shows nucleophilic attack to substrate, has been reported.[11,12] But an amide substrate was included in the above complexes as a mostly intramoleculer hydrolysis reaction took place without a catalytic cycle. Further, it has not been discussed about the effect of the carboxylate ligand on hydrolysis activity in model and enzymatic studies. We designed a new model complex for the active site of mono-nuclear zinc-peptidases providing (i) two imidazoles and one carboxylate which correspond to histidine and glutamic/aspartic acid, respectively (ii) coordinated water molecule which deprotonates to produce the nucleophile Zn-OH, and (iii) steric hindrance preventing polymerization. This paper reports hydrolysis reactions of amide by the designed mono-nuclear zinc-complex $[ZnL1(OH_2)]^-$ **1** and the effect of carboxylate ligand on hydrolysis activity by comparing with a model complex of *carbonic anhydrase* $[ZnL2(OH_2)]^{2-}$ **2**,[13] whose coordinated water has been well characterized in solution, where L1=bis(2-benzimidazolylmethyl)glycinate and L2=tris(2-benzimidazolylmethyl)amine.

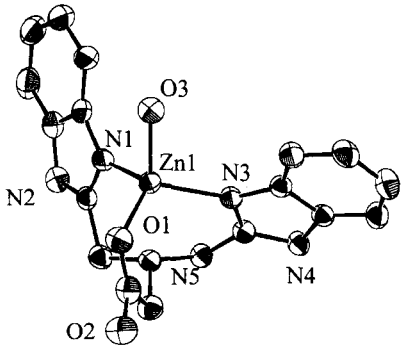


Figure 1. ORTEP drawing of molecular structure of **1**. Ellipsoids are depicted at the 30% probability level. Selected bond distances (Å): Zn1-O1 1.987(4), Zn1-O3 1.947(7), Zn1-N1 2.025(4), Zn1-N3 2.041(3), Zn1···N5 2.360(4).

Sodium salt of the new ligand NaL1[15] was synthesized from bis(2-benzimidazolylmethyl)amine,[14] bromoacetic acid, and sodium hydroxide. The model complex **1**·CF_3SO_3 was synthesized by a reaction of NaL with equimolar of $Zn(CF_3SO_3)_2$ in water.[16]

From the X-ray crystallographic data of **1**·$[ZnL1Cl]_3$·BF_4·$5H_2O$,[17] a zinc ion is coordinated by two imidazoles and one carboxylate of L1, and one water molecule or a chloride ion which occupies an apical position around zinc. The molecular structure of **1** shows tetrahedral geometry similar to that in native mono-nuclear zinc-peptidases,[1-6] as shown in Figure 1. Electrospray ionization (ESI) mass spectra showed that major species both in acetone and acetone/H_2O (9:1) are mono-nuclear complex (ZnL1) corresponding to solid state structure of Figure 1.

^{1}H NMR showed that the model complex **1** has the hydrolysis reactivity to amide of β-lactam to produce zinc-bound β-alanine (P1) and free β-alanine (P2) at 37 °C in acetone-d_6 (Figure 2) or acetone-d_6/D_2O (9:1, v/v, Figure 3). No hydrolysis of β-lactam showed without **1** in both solutions. Both α(P1)/β(P1) and α(P2)/β(P2) were assigned to the zinc-bound and freee β-alanines, respectively. Because the chemical shifts of β-alanine coordinated to zinc and free β-alanines showed the downfield and upfield shifts, respectively, as shown in Figure 2(b). Since the chemical shifts of β-lactam were constant in both solutions with and without **1**, β-lactam did not coordinate to zinc. The intensities of the methylene protons of β-lactam and coordinated water decreased with time in

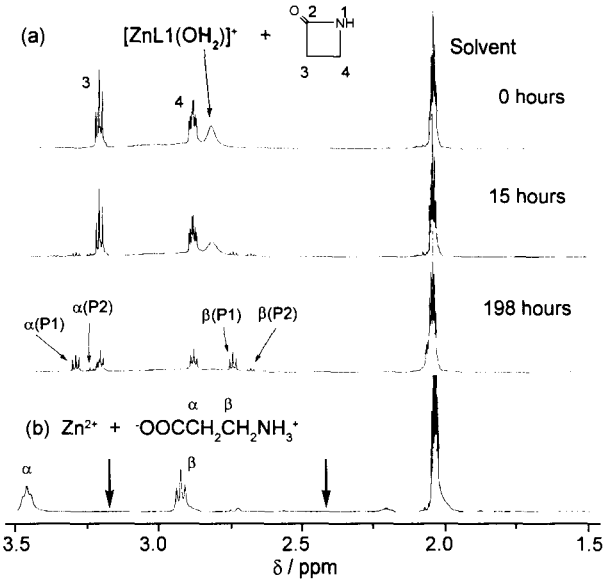


Figure 2. ^{1}H NMR spectra of (a) a solution of 50 mM **1** and 50 mM β-lactam in acetone-d_6 at 37 °C, and (b) a mixed solution of $Zn(CF_3SO_3)_2$ and β-alanine in acetone-d_6. The left and right arrows in (b) indicate the positions α and β of β-alanine in acetone-d_6/D_2O (9:1, v/v) without zinc complex.

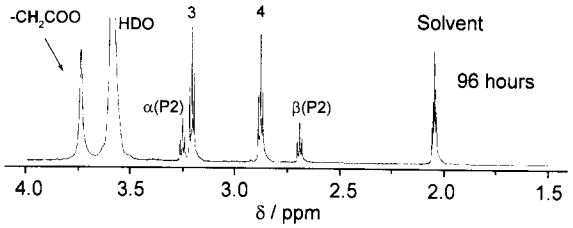
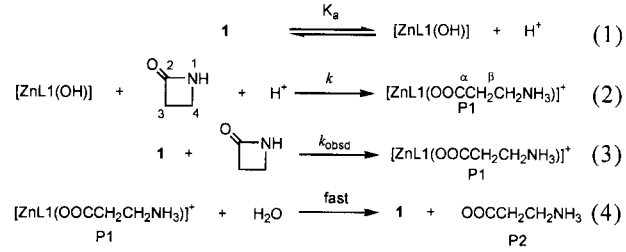


Figure 3. ^{1}H NMR spectra of a reaction solution of 50 mM **1** and 50 mM β-lactam in acetone-d_6/D_2O (9:1, v/v) at 37 °C.



acetone-d_6 at 37 °C (Figure 2), whereas new peaks α(P1)/β(P1) and α(P2)/β(P2) increased with time. This reaction proceeded quantitatively to produce P1 and P2 from the reactants β-lactam and coordinated water: total increase of P1 and P2 in intensity corresponded to decrease of β-lactam or coordinated water. Further, when NH proton of β-lactam (6.67 ppm) decreased, NH_3^+ group (1.28 ppm) of β-alanine increased as equimolar product. On the other hand, in acetone-d_6/D_2O (9:1, v/v), only free β-alanine α(P2)/β(P2) was observed in ^{1}H NMR (Figure 3). When there exists the excess of water, the concentration of which was about 100 times compared with that of zinc complex, a β-alanine in P1 is substituted by water molecule. Thus the model complex **1** showed catalytic cycle of hydrolysis in aqueous acetone solution.

Since the ligand-exchange reaction of eq. (4) is fast, the rate-determining step is eq. (2) also in acetone-d_6/D_2O solution. The observed reaction rate v_{obsd} was obtained from the decrease of the intensity of β-lactam in ^{1}H NMR and then observed rate constant, k_{obsd}, is written as follows

$$-d\,[\beta\text{-lactam}]\,/\,dt = v_{obsd} = k_{obsd}\,[\mathbf{1}][\beta\text{-lactam}] \quad (5)$$

$$v_{obsd,\,0} = k_{obsd}\,[\mathbf{1}]_0[\beta\text{-lactam}]_0 \quad (6)$$

k_{obsd} were obtained from eq. (6) and Figure 4 by initial slope

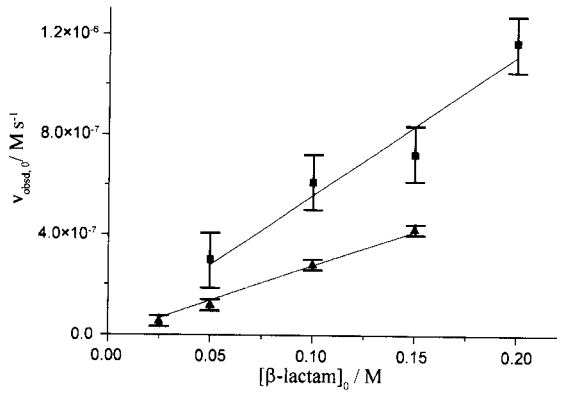


Figure 4. Plots of initial concentration of β-lactam versus observed initial rate; ■ ···acetone-d_6, ▲ ···acetone-d_6/D_2O (9:1, v/v).

Table 1. Second-order rate constants of hydrolysis reaction by model complexes

complex	solvent	k_{obsd} / M^{-1} s^{-1}
1	acetone-d_6	1.1×10^{-4}
	acetone-d_6/D_2O (9:1)	5.9×10^{-5}
2	acetone-d_6	——— [a]
	acetone-d_6/D_2O (9:1)	1.2×10^{-5}

[a]No hydrolysis reaction because of precipitate of zinc complex with L2 and β-lactam.

method (Table 1). The rate constant in acetone-d_6 is twice that in acetone-d_6/D_2O. The hydrolysis reaction between the nucleophile and β-lactam is disturbed by solvent water.

Since the rate constant of hydrolysis of β-lactam by the peptidase model **1** is five times as large as **2** in acetone-d_6/D_2O (9:1), as shown in Table 1, a role of the carboxylate ligand in **1** is to enhance the rate of hydrolysis of amide. This fact agrees with the prediction from ab initio calculations:[18] the HOMO energy at oxygen lone pair of zinc-bound hydroxide or water shows its own nucleophilicity enhanced by the negative carboxylate ligand. The hydrolysis reaction of β-lactam in this work may be model for hydrolysis of β-lactam ring in penicillins by β-lactamase.[19]

We are now in progress to design dinuclear zinc complex model for dinuclear zinc peptidases such as *leucine amino peptidase*[20] and investigate hydrolysis activity.

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15 Anal. Found: C, 57.67; H, 4.68; N, 18.66%. Calcd for $C_{18}H_{18}N_5O_3Na$ (NaL1 H_2O) : C, 57.59; H, 4.83; N, 18.65%. ^{1}H NMR (D_2O): 3.48(2H, s, CH_2), 4.10(4H, s, CH_2), 7.30-7.61(8H, m, benzimidazolyl). ^{13}C NMR (D_2O): 54.9(CH_2), 61.9(CH_2), 117.3, 125.3, 140.1, 155.8(benzimidazolyl), 181.4(carboxylate).
16 Anal. Found: C, 40.49; H, 3.34; N, 12.51%. Calcd for $C_{19}H_{18}N_5F_3O_6SZn$ (**1**·CF_3SO_3) :C, 40.26; H, 3.20; N, 12.36%.
17 The measurement was made on Rigaku AFC5R diffractometer (Cu K_α = 1.54178 Å). Crystal Data: **1**·[ZnL1Cl]$_3$·BF_4·5H_2O, $C_{36}H_{38}N_{10}O_7Cl_{1.5}B_{0.5}F_2Zn_2$, FW = 950.10, triclinic, $P\bar{1}$, a = 14.143(2) Å, b = 14.229(1) Å, c = 13.393(2) Å, α = 104.845(10)°, β = 117.90(1)°, γ = 77.594(10)°, V = 2288.0(6) Å^3, Z = 4, d_{calcd} = 1.379 g cm^{-1}, and R = 0.054.
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