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The Simulation Analysis of Dipeptide Hydrolysis Catalyzed by Divalent Metal Ions

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Some quantitative studies of the kinetics of peptide hydrolysis in the presence of metal ions have been hitherto recorded, and such divalent metal ions (M^{2+}) as Co(II)[1] and Cu(II)[2,3] have been pronounced as effective catalysts for the following reactions of dipeptide (A–B) hydrolysis:

$$\text{A–B}^{\pm} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{A}\smile\text{B} \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \text{B–A}^{\pm} \tag{1}$$

$$\text{A–B}^{\pm}(\text{or B–A}^{\pm}) \rightleftharpoons \text{A–B}^{-}(\text{or B–A}^{-}) + \text{H}^{+} \tag{2}$$

$$\text{A–B}^{-}(\text{or B–A}^{-}) + \text{M}^{2+} \rightleftharpoons \text{A–B--M}^{+}(\text{or B–A--M}^{+}) \tag{3}$$

$$\text{A–B--M}^{+}(\text{or B–A--M}^{+}) \underset{\text{H}_2\text{O}}{\overset{K}{\rightleftharpoons}} \text{M}^{2+} + \text{A} + \text{B} \tag{4}$$

where $\text{A}\smile\text{B}$ denotes the dioxopiperazine intermediate. Recently, Long *et al.*[4,5] have noted that the rate of glycyl-glycine hydrolysis is directly proportional to the concentrations of Co(II), Ni(II), Zn(II), and/or Mn(II) over the pH range of 3.8—6.0.

In the present paper, the simulation analysis was performed on the rate of dipeptide hydrolysis catalyzed by Zn(II), Co(II), and/or Ni(II) by means of a computer technique based on the kinetic method;[6] also the mechanism for the divalent metal ion-catalyzed dipeptide hydrolysis will be briefly discussed on the basis of ASMO-SCF and extended Hückel MO calculations.[7]

Results and Discussion

Let us first discuss the simulation results on Zn(II)-catalyzed hydrolyses of dipeptides; glycyl-L-leucine (G–L), glycyl-L-alanine (G–A), and glycyl-glycine (G–G). As can be seen from Fig. 1, the time conversions of the dipeptide concentrations calculated with the rate constants listed in Table 1 were satisfactorily in accordance with those[5] obtained experimentally. In the absence of the Zn(II) ion, only the acid H^+ (pH 5.6) accelerates a little the rate of G–L hydrolysis.

In regard to the cleavage of peptide bond in G–L G–A, and G–G, the order of the bond fission caused by the Zn(II) ion, G–G>G–A>G–L, was not directly related to that of the bond populations of their peptide bonds (G–G=1.036; G–A=1.035; G–L=1.035); this may be ascribed to the difference in the steric hindrances of the substituents (R_1 and R_2) in $H_3\overset{+}{N}CH(R_1)C(=O)NHCH(R_2)CO_2^-$ to the interaction of the Zn(II) ion.

Next, let us discuss the rate constant, k, of Reaction (3) in G–G hydrolysis with Zn(II), Co(II), and/or Ni(II). Fig. 2 shows the k values obtained experimentally[4] (k_{obsd}) and those calculated (k_{calcd}) by means of Arrhenius and activated complex theories on the basis of least-squares error analysis. Both the k_{obsd} and k_{calcd} values were found to be identical within the limits of technical error. As may be seen from Fig. 2, the Zn(II) ion seems to be predominant as a catalyst for the G–G hydrolysis. This distinctive catalytic nature of the Zn(II) ion is well reflected in the distinctive kinetic data of the Zn(II) ion (see Table 2).

Finally, the mechanism for the metal ion-catalyzed hydrolysis will be briefly discussed. The electronic structure of G–G in a weak acid solution, $H_3\overset{+}{N}CH_2C$-

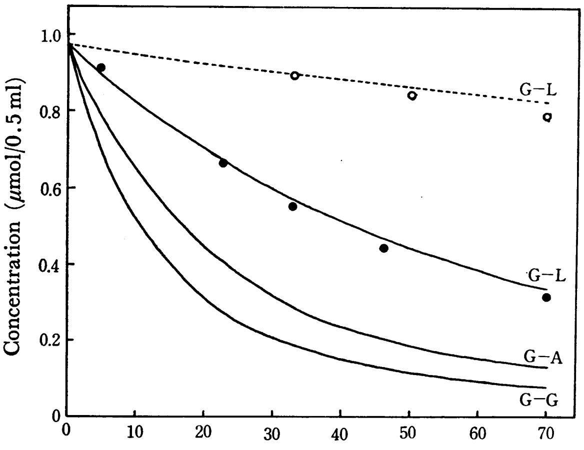

Fig. 1. Plots of dipeptide concentrations *vs.* reaction time in Zn(II) ion-catalyzed hydrolysis at 95 °C in pH 5.6. (dotted curve: absence of Zn(II); solid curve: presence of 0.04 M Zn(II); ○ and ●: experimental data cited from Ref. 5)

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[1] L. Lawrence and W. J. Moore, *J. Amer. Chem. Soc.*, **73**, 151 (1951).

[2] I. J. Grant and R. W. Hay, *Aust. J. Chem.*, **18**, 1189 (1965).

[3] M. M. Jones, T. J. Cook, and S. Brammer, *J. Inorg. Nucl. Chem.*, **28**, 1265 (1966).

[4] D. A. Long, T. G. Truscott, J. R. Cronin, and R. G. Lee, *Trans. Faraday Soc.*, **67**, 1094 (1971).

[5] J. R. Cronin, D. A. Long, and T. G. Truscott, *ibid.*, **67**, 2096 (1971).

[6] K. Ohkubo and T. Hashimoto, This Bulletin, **45**, 3350 (1972).

[7] The calculation details of the ASMO-SCF and the extended Hückel methods respectively have been described in the following references: R. Hoffmann, *J. Chem. Phys.*, **39**, 1397 (1963); *ibid.*, **40**, 2474 (1964) and T. Yonezawa, H. Konishi, and H. Kato, This Bulletin, **42**, 933 (1967). The Coulomb integrals of the C, N, and O atoms were taken from J. Hinze and H. H. Jaffé, *J. Amer. Chem. Soc.*, **85**, 540 (1963), except for that of the oxygen 2p-orbital (−15.85 eV).

Table 1. Second-order rate constants[a] used for the simulations of the reaction of glycine-containing dipeptides and dioxopiperazines in the presence of 0.04 M Zn(II) ion at pH 5.6 and 95 °C

Dipeptide	$10^3 k_1$	$10^3 k_{-1}$	$10^3 k_2$	$10^3 k_{-2}$	$10^3 k_{\mathrm{overall}}$[b]	$10^3 k'_{\mathrm{overall}}$[c]
G-G	17.5	12.5	17.5	12.5	11.8	11.8
G-A	11.25	4.0	6.25	3.0	6.25	7.25
G-L	3.75 (0.04)	1.5 (0.004)	4.5 (0.03)	1.5 (0.005)	3.25 (0.002)	3.25 (0.002)

a) Strictly speaking, these second-order rate constants can be represented by $k'(\mathrm{H}^+)$, wherein k' denotes third order rate constants. The values in parentheses indicate the rate constants for G-L hydrolysis in the absence of Zn(II).

b) The rate constant for the reaction, $\mathrm{A{-}B^{\pm}+M^{2+}+H_2O \rightarrow products}$.

c) The rate constant for the reaction, $\mathrm{B{-}A^{\pm}+M^{2+}+H_2O \rightarrow products}$.

Table 2. Kinetic data for the reaction of divalent metal ion-catalyzed G-G hydrolysis
$\mathrm{G{-}G{-}M^+ + H_2O \rightarrow products}$

Metal ion	Experimental[c]			Computer			
	A[a]	$\Delta S^{\ddagger}$ (cal/mol·K)	$\Delta H^{\ddagger}$ (kcal/mol)	A[a]	E[b] (kcal)	$\Delta S^{\ddagger}$ (cal/mol·K)	$\Delta H^{\ddagger}$ (kcal/mol)
Co(II)	5.79×10^{26}	+62	53	$(8.273\pm0.903)\times10^{26}$	54.01 ± 0.08	$+61.21\pm0.21$	53.27 ± 0.08
Ni(II)	5.30×10^{24}	+52	50	$(1.118\pm0.159)\times10^{25}$	51.30 ± 0.10	$+53.65\pm0.28$	50.56 ± 0.10
Zn(II)	9.93×10^{10}	−10	25	$(6.046\pm0.293)\times10^{10}$	25.42 ± 0.03	-11.52 ± 0.09	24.68 ± 0.03

a) Frequency factor, b) Activation energy, c) Cited from Ref. 4.

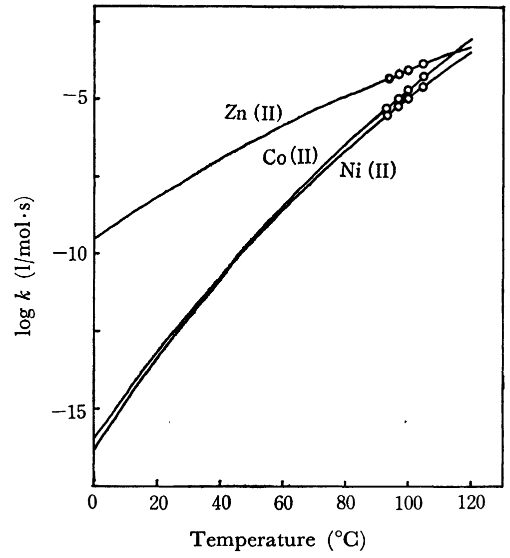


Fig. 2. Plots of log k *vs.* temperature in glycyl-glycine hydrolysis catalyzed by divalent metal ions. (○:experimental data cited from Ref. 4)

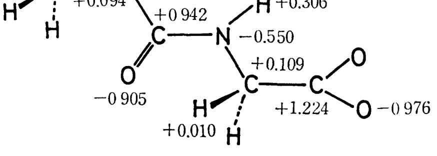
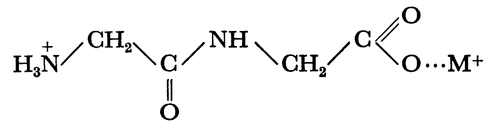


Fig. 3. Electronic structure of G-G in weak acid solution.

$\mathrm{({=}O)NHCH_2CO_2^-}$, is[8] presented in Fig. 3 by the use of the formal charges calculated by the ASMO-SCF method: the highest occupied (HO) MO of $\mathrm{G{-}G^{\pm}}$ is strongly localized by the nonbonding lone-pair oxygen 2p-orbital of the $\mathrm{CO_2^-}$ group. Bearing in mind that the divalent metal ion behaves as an electron acceptor against $\mathrm{G{-}G^{\pm}}$, an assumed complex, $\mathrm{A{-}B\cdots M^+}$ (or $\mathrm{B{-}A\cdots M^+}$), in Eqs. (2) and (3) may be represented by the following interacting system:



$$\mathrm{H_3\overset{+}{N}{-}CH_2{-}\underset{\underset{\displaystyle O}{\|}}{C}{-}NH{-}CH_2{-}C\overset{\displaystyle /\!\!/ O}{}\!\!\!\diagdown O\cdots M^+}$$

The distinctive nature of the Zn(II) ion mentioned above may be the result of the low-lying, lowest-unoccupied (LU) AO level of the Zn(II) ion; In view of the orbital energies of the LU AOs of the metal ions (−9.39 eV[9]) for the Zn(II) p-orbital; −7.86 eV[9]) for the Co(II) d-orbital; −7.633 eV[9]) for the Ni(II) d-orbital) and the HOMO of the nonbonding lone-pair 2p-orbital of oxygen (−10.50 eV) in the terminal $\mathrm{CO_2^-}$ group, it seems from the energetic point of view[10] that the Zn(II) ion may interact easily and strongly with $\mathrm{G{-}G^{\pm}}$ in the above interacting system. Therefore, the nucleophilic attacking of the $\mathrm{H_2O}$ molecule on the more positively-charged carbonyl carbon in the peptide bond may be expected in the case of $\mathrm{G{-}G{-}Zn^+}$.

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[8] With regard to the geometry of glycyl-glycine (Zwitterion), see L. E. Sutton, ed., "Interatomic Distances," The Chemical Society, London (1958).

[9] C. E. Moore, "Atomic Energy Levels," Natl. Bur. Std. Circ., No. 467 (1958), Vols. 1, 2, and 3.

[10] In an aqueous solution, the divalent metal ion may react with dipeptides as an aquo-complex involving a fixed number of water molecules.[11] Therefore, we should take into consideration the lability order of aquo-complexes in the water substitution with the dipeptide. In this respect, the lability order, Zn(II) ≫ Co(II) > Ni(II),[12] and/or the order of the rates in water-molecule exchange, Co(II) ≥ Ni(II),[13] are closely related to that of the rates in the dipeptide hydrolyses by the divalent metal ions.

[11] H. Taube, "Progress in Stereochemistry," Butterworths, Washington, D. C. (1962), Vol. 3, p. 95.

[12] G. Geier, *Bunsenges. Phys. Chem.*, **69**, 617 (1965).

[13] T. J. Swift and R. E. Connick, *J. Chem. Phys.*, **37**, 307 (1962).