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KINETICS OF STEREOSELECTIVE COMPLEXATION REACTIONS OF HISTIDINE AND LEUCYLTYROSINE WITH DIVALENT METAL IONS IN AQUEOUS SOLUTION

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The optical activity of amino acids or peptides influences the chemical equilibrium. Bis histidine complex ML_2 and MLD differ in the stability in aqueous solution (M^{2+} is the metal ion and D^-, L^- are the L and D forms of histidine anion). Dipeptides, L-leucyl-L-tyrosine and D-leucyl-L-tyrosine, differ in the pK values and the stabilities of complexes with metal ions. The complexation reactions of these stereoselective systems were studied kinetically by the temperature jump method.

On histidine complex systems, the rate constants of these types were obtained.

$$ML^+ + L^- \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ML_2,$$

$$ML^+ + D^- \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} MLD.$$

At 25°C and ionic strength of 0.1, rate constants are: nickel (II), $k_2 = 4.1 \times 10^5\,M^{-1}\,sec^{-1}$, $k_{-2} = 0.059\,sec^{-1}$, $k_3 = 4.3 \times 10^5\,M^{-1}\,sec^{-1}$, $2k_{-3} = 0.027$; cobalt (II), $k_2 = 1.4 \times 10^6\,M^{-1}\,sec^{-1}$, $k_{-2} = 5.7\,sec^{-1}$, $k_3 = 1.2 \times 10^5\,M^{-1}\,sec^{-1}$, $2k_{-3} = 2.9\,sec^{-1}$; zinc (II), $k_2 = 6.0 \times 10^7\,M^{-1}\,sec^{-1}$, $k_{-2} = 190\,sec^{-1}$, $k_3 = 5.2 \times 10^7\,M^{-1}\,sec^{-1}$, $2k_{-2} = 96\,sec^{-1}$; cadmium (II), $k_2 \approx 7 \times 10^7\,M^{-1}\,sec^{-1}$, $k_{-2} \approx 4 \times 10^3\,sec^{-1}$ (The coefficient 2 of k_{-3} is the statistical factor). The formation rate constants k_2 and k_3 are equal within experimental error for nickel (II), cobalt (II) and zinc (II), but $2k_{-3}$ is about half of k_{-2}.

On leucyltyrosine systems, the rate constants of the following types were obtained.

$$Ni^{2+} + A^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} NiA^+,$$

$$NiA^+ + A^- \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} NiA_2,$$

where A^- is the optical active dipeptide anion. The results at 25°C and ionic strength of 0.15 are: L-leucyl-L-tyrosine $k_1 = 2.2 \times 10^3\,M^{-1}\,sec^{-1}$, $k_{-1} = 1.3\,sec^{-1}$, $k_2 = 1.7 \times 10^3\,M^{-1}\,sec^{-1}$, $k_{-2} = 3.0\,sec^{-1}$; D-leucyl-L-tyrosine, $k_1 = 2.4 \times 10^3\,M^{-1}\,sec^{-1}$, $k_{-1} = 0.45\,sec^{-1}$, $k_2 = 2.0 \times 10^3\,M^{-1}\,sec^{-1}$, $k_{-2} = 2.4\,sec^{-1}$. The value of k_1 for L-leucyl-L-tyrosine is equal within experimental error to that for D-leucyl-L-tyrosine, but in dissociation reactions the value of k_{-1} for D-leucyl-L-tyrosine is about one third of the value for L-leucyl-L-tyrosine.

The effect of the optical activity of ligands influences kinetically the dissociation reaction of the complex. The fact that there is no effect on the rate

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constant of the formation reaction indicates that the rate-determining step is the first unidentate complex formation step of the incoming ligand involving the water dissociation from the inner hydration shell of the metal ion *via* diffusion-controlled ion-pair formation.

Introduction

It is well known that the optical activity of ligands in chelate complexes influences the chemical equilibrium and the reaction in solution. In ester hydrolysis, the catalytic effect of metal complexes are influenced by the optical activity of ligands[1,2]. In the dimerization reaction of metal complex, the effect of the optical activity of ligands on the reaction rates were observed[3,4]. But the quantitative kinetic study of stereoselectivity on complex formation and dissociation reactions were not sufficiently carried out. The thermodynamic effect of the optical activity of ligands is known from the stability constants of complexes in solution. Ritsma *et al.*[5] studied the stability constants of Co(II) and Ni(II) complexes of asparagine, gultamine, aspartic acid and glutamic acid, Cu(II) complexes of asparagine and glutamine using L and DL amino acids. But no difference was observed in L and DL amino acids. In Co(II)-L-histidine and Co(II)-DL-histidine systems, Mcdonald and Phillips[6] found, by the NMR method, that Co(L-His)(D-His) is more stable than Co(L-His)$_2$ or Co(D-His)$_2$. Ritsma *et al.*[7] determined the stability constants of Ni(II) and Co(II) complexes of L-histidine and racemic histidine by the potentiometric method and found the stereoselective effect. Morris *et al.*[8] found the same effect in Co(II), Ni(II) and Zn(II) complexes of histidine, but Cd(II) and Cu(II) complexes had no stereoselective effect.

The stereoselective complexation was found in L-leucyl-L-tyrosine and D-leucyl-L-tyrosine complexes with divalent metal ions by Li *et al.*[9].

These dipeptides differ in the pK values and stability constants with metal ions.

As the equilibrium constants of the complex formation-dissociation reaction is a ratio of the forward and backward rate constants, a stereoselective effect in equilibrium systems originates in the difference of rate constants. In this investigation, the kinetics of stereoselective complexation reactions were studied by the temperature jump method.

Experimental

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L-histidine and DL-histidine were recrystallized from water-ethanol. The purity was checked by the paper-chromatopraphy and the optical rotation.

Reagent grade nickel nitrate, cobalt nitrate, zinc sulfate and cadmium sulfate were used without further purification and the stock solutions of the metal ions were determined using the EDTA titration method. Phenol red and cresol red were used as indicators. All solutions were prepared with freshly boiled distilled water and brought to ionic strength of 0.1 with reagent grade KNO_3 or KCl. The pH of the solution was measured with a Horiba F5 pH meter at 25°C. The hydrogen ion concentration of the solution was calculated by the activity coefficient of 0.772 from the pH value. As the bis histidine complex of Co (II) makes an oxygen absorbed dimer which may disturb complexation reactions, the sample solutions were used under nitrogen[8].

In practice, without high concentration solutions of the complex, any significant difference between the measured results under the air and the nitrogen atmosphere conditions did not appear.

The experimental procedures and instrumentation were described in a previous paper[10]. The 0.1 or 0.6 μF condencer was charged to 8~13 kV and then discharged through 0.5 or 1.0 m*l* observation cell. The temperature of the observation cell was controlled to be 25°C after the temperature jump.

In every time, only single relaxation was observed. No relaxation was observed for indicator-amino acid or indicator-metal ion solutions. Each relaxation time was obtained as the average of at least four photographic determinations. The equilibrium concentration of each solution was calculated using the equilibrium constants at Table 1.

Table 1 Equilibrium constants of metal ion-histidine systems at 25°C and ionic strength 0.1

Metal ion	Ligand	K_1	K_2	K_3
Ni (II)	L-histidine	5.53×10^8	6.93×10^6	
Ni (II)	DL-histidine	4.42×10^8		3.18×10^7
Co (II)	L-histidine	7.31×10^6	2.45×10^5	
Co (II)	DL-histidine	7.33×10^6		8.24×10^5
Zn (II)	L-histidine	3.63×10^6	3.16×10^5	
Zn (II)	DL-histidine	3.39×10^6		1.08×10^6
Cd (II)	L-histidine	2.45×10^5	1.86×10^4	

$K_1=(ML^+)/(M^{2+})(L^-)$, $K_2=(ML_2)/(ML^+)(L^-)$, $K_3=(MLD)/(ML^+)(D^-)$
$pK_{L1}=1.645$,
$pK_{L2}=6.037$ (Ni (II), Co (II), 0.15 M, KNO_3), 6.14 (Zn (II), Cd (II), 0.1 M, KCl),
$pK_{L3}=9.097$ (Ni (II), Co (II), 0.1M, KNO_3), 9.20 (Zn (II), Cd (II), 0.1 M, KCl)
$pK_1=7.9$ (phenol red), 8.1 (cresol red)

L-leucyl-L-tyrosine and D-leucyl-L-tyrosine were the products of Institute for Protein Reseach (Osaka). Complexation kinetics of these dipeptides with Ni (II) were studied by the above mentioned temperature jump method.

No discernible relaxation was obtained when Co (II) or Zn (II) was used instead of Ni (II). Each

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relaxation time was obtained as the average of at least six photographic determinations. The relative error for these relaxation measurement is within ±15%.

Results

Ni (II)-L-histidine system

The following complex formation-dissociation reactions exist in the solution:

$$M^{2+}+L^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ML^+, \qquad (1)$$

$$ML^+ + L^- \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ML_2, \qquad (2)$$

where M^{2+} and L^- represent the metal ion and the ligand anion, respectively. The rate constants are shown as k_1, k_{-1}, k_2 and k_{-2}. Except complexation reactions (1) and (2), the fast protolytic reactions of the ligand and the indicator exist in the solution.

$$H^+ + L^- \rightleftharpoons HL, \quad K_{L3} \qquad (3)$$

$$H^+ + In^- \rightleftharpoons HIn, \quad K_I \qquad (4)$$

In^- represents the dissociated state of the indicator. K_{L3} and K_I are the dissociation constants of histidine and the indicator, respectively.

As protolytic reactions (3) and (4) reach equilibrium much faster than the metal complex formation-dissociation reactions, they can be assumed to be at equilibrium at all times. The equilibrium concentrations and observed relaxation times of Ni(II)-L-histidine systems are shown in Table 2. The equilibrium concentrations of Ni^{2+} are much smaller than those of NiL^+ or NiL_2, so the contribution of reaction (1) to the observed relaxation times may be neglected. The reciprocal relaxation time referred

Table 2 Relaxation times and equilibrium concentrations of Ni (II)-L-histidine solutions

$(Ni^{2+})_0$ $(10^{-3}M)$	$(L^-)_0$ $(10^{-3}M)$	pH	(Ni^{3+}) $(10^{-4}M)$	(NiL^+) $(10^{-4}M)$	(NiL_2) $(10^{-4}M)$	(L^-) $(10^{-6}M)$	τ (msec)
0.987	2.01	7.24	0.001	0.839	9.03	1.55	100
0.987	1.95	7.51	0.001	0.858	9.01	1.52	104
1.97	3.99	7.21	0.001	1.26	18.4	1.12	45
1.97	4.08	7.28	0.001	0.850	18.9	3.20	55
2.96	5.96	7.50	0.001	0.13	28.5	3.65	44
3.95	8.17	7.59	0.000	0.582	38.9	9.65	44
3.65	7.88	7.52	0.001	1.58	37.9	3.46	38
5.92	11.6	7.42	0.003	3.47	55.7	2.32	21
5.92	12.1	7.33	0.000	1.26	57.9	66.5	30
5.92	11.9	7.32	0.001	1.89	57.3	4.37	23

$(Ni^{2+})_0$ and $(L^-)_0$ indicate the initial concentrations of metal ion and ligand, respectively. Phenol red was used as indicator (initial concentration $(In)_0 = 2.06 \times 10^{-5}M$).

to reactions (2), (3) and (4) is[11],

$$\frac{1}{\tau}=k_2\left[\frac{(ML^+)}{(1+\alpha)}+(L^-)\right]+k_{-2},$$

$$=k_2\left[\frac{(ML^+)}{(1+\alpha)}+(L^-)+\frac{1}{K_2}\right], \qquad (5)$$

$$\alpha=\frac{(H^+)}{K_{L3}+(L^-)\beta}, \qquad \beta=\frac{K_I+(H^+)}{K_I+(H^+)+(In^-)},$$

where τ is the relaxation time and (ML^+), *etc.* are the equilibrium concentrations. K_2 is the complex formation constant. The value of k_2 is obtained from the plot of $1/\tau$ *vs* $(ML^+)/(1+\alpha)+(L^-)+1/K_2$.

This plot is shown in Fig. 1. The value of k_2 is $4.1\times10^5\,M^{-1}sec^{-1}$ and k_{-2} is determined from k_2/K_2.

If both the anion L^- and zwitter ion HL are involved in the reaction mechanism, the next reaction must be added to reaction (2).

$$ML^+ + HL \underset{k'_{-2}}{\overset{k'_2}{\rightleftharpoons}} ML_2 + H^+. \qquad (6)$$

The reciprocal relaxation times considering reactions (2), (3), (4) and (6) are shown in the following equation[12]:

$$\frac{1}{\tau}=k_2AA+k'_2BB, \qquad (7)$$

$$AA=\frac{(ML^+)}{1+\alpha}+(L^-)+\frac{1}{K_2},$$

$$BB=\frac{(ML^+)}{1+\alpha}+(HL)+\frac{1}{K_2K_3(H^+)+\alpha\beta}\frac{(ML_2)}{1+\alpha}.$$

The plot of $1/(\tau BB)$ *vs.* AA/BB is shown in Fig. 2. The slope of this line is k_2 and the intercept is k'_2. From the plot, k'_2 is ≈0.

The value of k_2 coincides with the value obtained from Fig. 1. HL does not contribute to the complexation reaction directly. It is a general behavior that the reactivity of the zwitter ion HL of

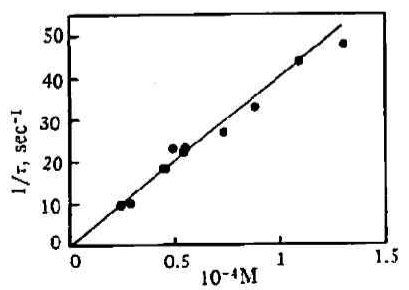

Fig. 1 Plot of $1/\tau$ *vs.* $(NiL^+)/(1+\alpha)+(L^-)+1/K_2$ Ni (II)-L-histidine solution

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amino acids is very small compared to the dissociated ion L^-[13,14].

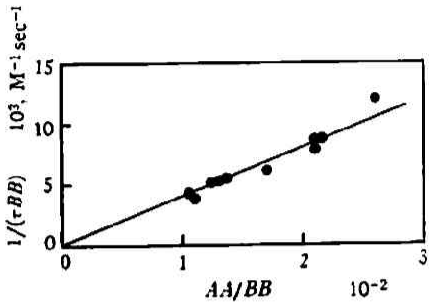


Fig. 2 Plot of $1/(\tau BB)$ *vs.* AA/BB Ni (II)-L-histidine solution

Ni (II)-DL-histidine systems

Equilibrium concentrations and relaxation times are listed in Table 3 and the representative relaxation spectra of Ni (II)-DL-histidine solution is shown in Fig. 3. As the mixed complex MLD is more stable than ML_2, the relationship of equilibrium concentrations in the absence of stereoselectivity, does not hold in this system, where L^- and D^- are L- and D-histidine anions, respectively.

$$(MLD)=(ML_2)+(MD_2)=2(ML_2)\,, \qquad (8)$$

Table 3 Relaxation times and equilibrium concentrations of Ni (II)-DL-histidine solutions

$(Ni^{2+})_o$ (10^{-3}M)	$(L^-)_o$ (10^{-3}M)	pH	(Ni^{2+}) (10^{-4}M)	$2(NiL^+)$ (10^{-4}M)	$2(NiL_2)$ (10^{-4}M)	(NiLD) (10^{-4}M)	$2(L^-)$ (10^{-6}M)	τ (msec)
0.987	2.16	7.30	0.000	0.256	2.91	6.67	2.28	125
1.97	3.85	7.20	0.004	1.69	5.47	12.5	0.534	47
1.97	3.90	7.49	0.001	0.867	5.66	13.0	1.95	91
2.96	5.71	7.25	0.007	2.64	8.15	18.7	0.860	42
2.92	5.89	7.38	0.002	1.33	8.58	19.7	1.86	47
3.95	7.55	7.30	0.011	4.00	10.8	24.7	0.778	32
3.95	7.73	7.25	0.005	2.62	11.2	25.7	1.23	38
4.94	10.0	7.44	0.000	0.893	14.7	33.8	4.76	49
4.94	9.83	7.21	0.003	2.24	14.3	32.9	1.85	26
5.92	11.8	7.12	0.002	2.11	17.3	39.8	2.36	26

Phenol red was used as indicator ($(In)_o = 2.06\times10^{-5}$M).

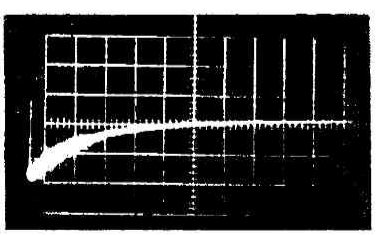


Fig. 3 Relaxation spectrum for Ni (II)-DL-histidine solution
$(Ni^{2+})_o = 2.96\times10^{-3}$M, $(L^-)_o = 5.89\times10^{-3}$M, pH 7.38
The abscissa scale is 20 msec per major division.

13) R. F. Pasternack, E. Gibbs and J. C. Cassatt, *J. Phys. Chem.*, **73**, 3814 (1969)
14) J. C. Cassatt and R. G. Wilkins, *J. Am. Chem. Soc.*, **90**, 6045 (1968)

The following equilibrium exists in the solution:

$$\left.\begin{aligned} &M^{2+}+L^- \underset{k_{-L}}{\overset{k_L}{\rightleftharpoons}} ML^+, \\ &M^{2+}+D^- \underset{k_{-D}}{\overset{k_D}{\rightleftharpoons}} MD, \end{aligned}\right\} \tag{9}$$

$$\left.\begin{aligned} &ML^++L^- \underset{k_{-LL}}{\overset{k_{LL}}{\rightleftharpoons}} ML_2, \\ &MD^++D^- \underset{k_{-DD}}{\overset{k_{DD}}{\rightleftharpoons}} MD_2, \end{aligned}\right\} \tag{10}$$

$$\left.\begin{aligned} &ML^++D^- \underset{k_{-LD}}{\overset{k_{LD}}{\rightleftharpoons}} MLD, \\ &MD^++L^- \underset{k_{-DL}}{\overset{k_{DL}}{\rightleftharpoons}} MLD, \end{aligned}\right\} \tag{11}$$

$$\left.\begin{aligned} &H^++L^- \rightleftharpoons HL, \quad K_L \\ &H^++D^- \rightleftharpoons HD, \quad K_D \\ &H^++In^- \rightleftharpoons HIn, \quad K_I \end{aligned}\right\} \tag{12}$$

where K_L and K_D are the dissociation constants of L-histidine and D-histidine, respectively. As L^- and D^- differ only in spatial symmetry, the rate constants are equal with L^- and D^-, if the interaction energy between the reaction partners is equal in the course of the reaction. Therefore, the rate constants and equilibrium constants in (9)~(12) are arranged as follows:

$$\left.\begin{aligned} &k_L=k_D=k_1, \\ &k_{-L}=k_{-D}=k_{-1}. \end{aligned}\right\} \tag{13}$$

$$\left.\begin{aligned} &k_{LL}=k_{DD}=k_2, \\ &k_{-LL}=k_{-DD}=k_{-2}, \end{aligned}\right\} \tag{14}$$

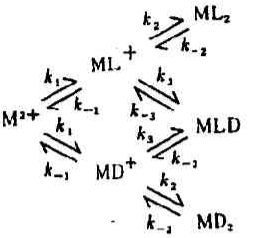
$$\left.\begin{aligned} &k_{LD}=k_{DL}=k_3, \\ &k_{-LD}=k_{-DL}=k_{-3}, \end{aligned}\right\} \tag{15}$$

$$K_L=K_D=K_{L3}, \tag{16}$$

and the equilibrium concentration relations are shown as follows:

$$\left.\begin{aligned} &(L^-)=(D^-), \qquad (HL)=(HD), \\ &(ML^+)=(MD^+), \quad (ML_2)=(MD_2), \end{aligned}\right\} \tag{17}$$

The overall reaction scheme except acid-base equilibrium are shown as follows:



The relation between the relaxation times and equilibrium concentrations was deduced by straightforward calculations,

$$\begin{vmatrix} a_{22}-1/\tau & a_{23} \\ a_{32} & a_{33}-1/\tau \end{vmatrix}=0.$$

$1/\tau$ is represented as follows:

$$\frac{1}{\tau_\pm}=\frac{1}{2}\left[a_{22}+a_{33}\pm\sqrt{(a_{22}+a_{33})^2-4(a_{22}a_{33}-a_{23}a_{32})}\right], \tag{18}$$

where

$$a_{22}=k_2\left[\frac{(ML^+)}{1+\alpha}+(L^-)+\frac{1}{K_2}\right], \qquad a_{23}=-k_2\left[\frac{(ML^+)}{1+\alpha}+(L^-)\right],$$

$$a_{32}=2k_3\left[\frac{(ML^+)}{1+\alpha}+(L^-)\right], \qquad a_{33}=k_3\left[\frac{(ML^+)}{1+\alpha}+(L^-)+\frac{2}{K_3}\right],$$

$$\alpha=\frac{(H^+)}{K_{L3}+2(L^-)\dfrac{K_1+(H^+)}{K_1+(H^+)+(In^-)}}.$$

Although Eq. (18) contains unknown variables, k_2 and k_3, the value of k_2 is known in the analysis of Ni (II)-L-histidine system.

The value of k_2 was introduced into Eq. (18) and $1/\tau_\pm$ was calculated using the trial values of k_3. The value of k_3 was chosen to make the following equation minimum:

$$\sum\left(\frac{1/\tau_{obs}-1/\tau_{calc}}{1/\tau_{obs}}\right)^2. \tag{19}$$

The value of $1/\tau_{obs}$ is τ_+ in Eq. (18). τ_- did not fit to the observed values. The summation of Eq. (19) is taken for the experimental number. The calculated value of k_2 was $4.2\times10^5\,M^{-1}\,sec^{-1}$.

The value of k_3 was also obtained graphically, simplifying the terms a_{22} and a_{33}. The term a_{22} is the sum of $(ML^+)/(1+\alpha)+(L^-)$ and $1/K_2$.

The value of $(ML^+)/(1+\alpha)+(L^-)$ is the order of 10^{-5} but the value of $1/K_2$ is 1.4×10^{-7}. Therefore, $1/K_2$ is negligible compared with $(ML^+)/(1+\alpha)+(L^-)$, then Eq. (18) is simplified as

$$\left.\begin{aligned}\frac{1}{\tau_+}&=(k_2+k_3)\left[\frac{(ML^+)}{1+\alpha}+(L^-)\right],\\ \frac{1}{\tau_-}&=0.\end{aligned}\right\} \tag{20}$$

The plot of $1/\tau$ *vs.* $2[(ML^+)/(1+\alpha)+(L^-)]$ is shown in Fig. 4. The value of $(k_2+k_3)/2$ is obtained

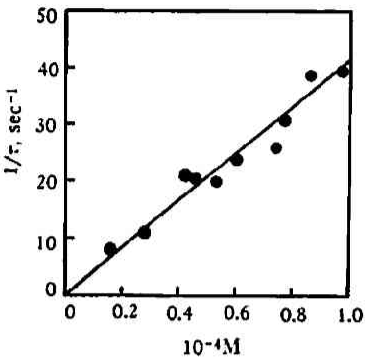


Fig. 4 Plot of $1/\tau$ *vs.* $2[(NiL^+)/(1+\alpha)+(L^-)]$ Ni (II)-DL-histidine solution

from the plot of Fig. 4. The value of $(k_2+k_3)/2$ is $4.2\times10^5\,M^{-1}\,sec^{-1}$ and that of k_3 is $4.3\times10^5 M^{-1}$ sec^{-1}. The values of k_3 obtained from the numerical and graphical method are in a reasonable agreement.

Co (II)-L-histidine and Co (II)-DL-histidine systems

Experimental conditions and observed relaxation times for Co (II)-L-histidine systems are listed in Table 4. The plot of $1/\tau$ *vs.* $(ML^+)/(1+\alpha)+(L^-)+1/K_2$ is shown in Fig. 5. The value of k_2 from the slope is $1.4\times10^6\,M^{-1}sec^{-1}$. The plot of $1/(\tau BB)$ *vs.* AA/BB is shown in Fig. 6. From this plot, the value of k'_2 is nearly zero, similar to the case of Ni (II)-L-histidine system. The experimental conditions

Table 4 Relaxation times of Co (II)-L-histidine solutions

$(Co^{2+})_t$ $(10^{-3}M)$	$(L^-)_t$ $(10^{-3}M)$	$(In)_t$ $(10^{-5}M)$	pH	τ (msec)
0.504	1.94	1.99	8.18	4.5
0.973	1.92	2.00	7.55	3.2
0.973	4.09	2.00	7.69	4.3
0.973	2.04	1.90	8.33	6.4
0.973	2.00	1.94*	7.74	4.3
1.07	2.32	2.06	7.21	3.2
1.07	1.91	2.06	7.14	3.2
1.95	6.40	2.00	7.62	3.0
1.95	5.19	2.00	7.21	3.4
1.95	4.57	1.94*	8.11	3.0
2.14	4.35	1.99	8.05	2.5
3.21	6.07	1.99	7.66	1.1
3.89	4.00	2.00	7.20	1.2
3.89	7.77	2.00	8.34	2.1
3.89	7.76	1.94*	7.90	1.3
5.84	11.6	2.00	8.22	1.4

Phenol red was used as indicator except those with * (cresol red).

and the observed relaxation times for Co (II)-DL-histidine systems are shown in Table 5, and the plot based on Eq. (20) is shown in Fig. 7.

Table 5 Relaxation times of Co (II)-DL-histidine solutions

$(Co^{2+})_0$ (10^{-3}M)	$(L^-)_0$ (10^{-3}M)	$(In)_0$ (10^{-5}M)	pH	τ (msec)
0.504	1.14	1.30	7.86	4.0
0.973	2.43	2.00	7.26	4.5
1.07	1.99	1.30	7.74	2.8
1.07	1.95	1.30	7.25	3.4
1.95	3.83	2.00	7.92	3.0
1.95	3.86	1.94	7.67	2.8
2.14	4.07	1.30	8.16	2.3
3.21	6.14	1.30	8.34	2.5
3.21	5.79	1.30	8.14	1.6
3.89	8.26	2.11	8.17	2.6
5.84	10.8	2.11	7.67	0.96
7.78	16.5	1.94	8.28	2.5

The value of k_3 is determined to be $1.2\times10^6 M^{-1}sec^{-1}$. The value of k_3 from the numerical calculation of relaxation times using Eqs. (18) and (19) coincides with that from the graphical method.

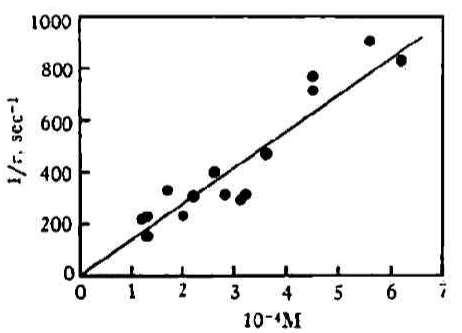


Fig. 5 Plot of $1/\tau$ *vs.* $(CoL^+)/(1+\alpha)+(L^-)+1/K_2$ Co (II)-L-histidine solution

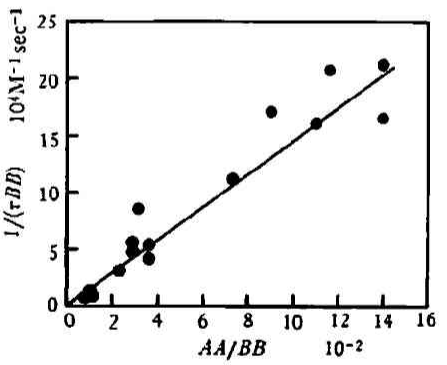


Fig. 6 Plot of $1/(\tau BB)$ *vs.* AA/BB Co (II)-L-histidine solution

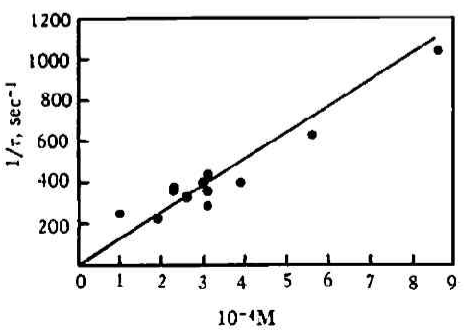


Fig. 7 Plot of $1/\tau$ *vs.* $2[(CoL^+)/(1+\alpha)+(L^-)]$ Co (II)-DL-histidine solution

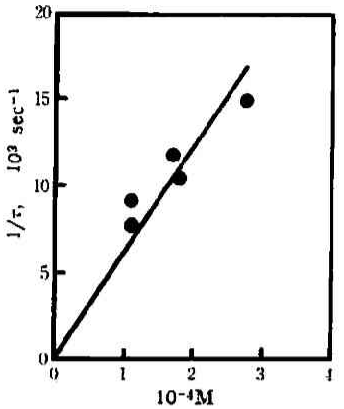


Fig. 8 Plot of $1/\tau$ *vs.* $(ZnL^+)/(1+\alpha)+(L^-)+1/K_2$ Zn (II)-L-histidine solution

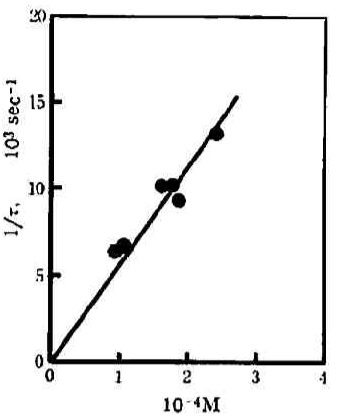


Fig. 9 Plot of $1/\tau$ *vs.* $2[(ZnL^+)/(1+\alpha)+(L^-)]$ Zn (II)-DL-histidine solution

Table 6 Relaxation times of Zn (II)-L-histidine, Zn (II)-DL-histidine and Cd (II)-L-histidine solutions

$(M^{2+})_o$ (10^{-3}M)	$(L^-)_o$ (10^{-3}M)	$(In)_o$ (10^{-5}M)	pH	τ (μsec)
		Zn (II)-L-histidine solutions		
0.500	1.02	2.00	7.49	110
0.500	1.21	2.00	7.46	130
1.00	2.41	2.00	7.44	96
1.00	1.99	2.00	7.72	85
2.00	4.36	2.00	7.68	68
		Zn (II)-DL-histidine solutions		
0.500	1.16	2.00	7.43	160
0.500	1.02	2.00	7.29	150
1.00	2.03	2.03	7.40	120
1.00	2.10	1.99	7.41	95
1.00	2.07	1.99	7.57	96
2.00	4.01	2.00	7.75	76
		Cd (II)-L-histidine solutions		
0.153	0.529	1.99	7.37	140
0.305	0.696	1.99	7.87	62

Table 7 Rate constants of complexation reactions of metal ions with histidine

	k_2 ($M^{-1}sec^{-1}$)	k_3 ($M^{-1}sec^{-1}$)	k_{-2} (sec^{-1})	$2k_{-3}$ (sec^{-1})
Ni (II)	4.1×10^5	4.3×10^5	5.9×10^{-2}	2.7×10^{-2}
Co (II)	1.4×10^6	1.2×10^6	5.7	2.9
Zn (II)	6.0×10^7	5.2×10^7	190	96
Cd (II)	$\simeq7\times10^7$		$\simeq4\times10^3$	

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Zn (II)-L-histidine, Zn (II)-DL-histidine and Cd (II)-L-histidine systems

The complexation reactions of Zn (II) and Cd (II) are generally very fast and the observed relaxation signals were small. Therefore, the measurement for relaxation times in the wide concentration range was restricted.

The experimental conditions and the observed relaxation times are shown in Table 6, and the plots for Zn (II)-L-histidine and Zn (II)-DL-histidine systems are shown in Figs. 8 and 9, respectively. The rate constants were obtained from these plots.

Only two relaxation measurements were carried out for Cd (II)-L-histidine system. The rate constant k_2 was estimated using Eq. (5) and the observed relaxation times in Table 6. The rate constants of the above mentioned metal ion-histidine systems are compiled in Table 7.

Ni (II)-L-leucyl-L-tyrosine and Ni (II)-D-leucyl-L-tyrosine systems

The equilibrium constants for complexation reactions which were determined by Li *et al.*[9] are listed in Table 8 and the experimental conditions and the observed relaxation times are shown in Tables 9 and 10. Discernible relaxations could not be seen in Co (II) or Zn (II)-ligand-indicator solutions. Therefore, only Ni (II)-ligand-indaictor systems were studied in this investigation. The complexation reactions of types (1) and (2) are analyzed using fast protolytic reactions (3) and (4). The reciprocal relaxation times are,

$$\frac{1}{\tau_\pm}=\frac{1}{2}\left[a_{11}+a_{22}\pm\sqrt{(a_{11}+a_{22})^2-4(a_{11}a_{22}-a_{12}a_{21})}\right]. \tag{21}$$

a_{ii} is represented by the rate constants and the equilibrium concentrations[11].

Eq. (21) was used to calculate the relaxation times for a number of the trial values of the rate constants k_1 and k_2. The rate constants were chosen to give the best fit to all observed relaxation times

Table 8 pK values and formation constants with nickel (II) of L-leucyl-L-tyrosine and D-leucyl-L-tyrosine at 25°C and ionic strength 0.15 (KNO_3)[9]

	pK_A (COOH)	pK_A (NH^+_3)	pK_A (OH)	$\log K_1$ (Ni^{2+})	$\log K_2$ (Ni^{2+})
L-Leucyl-L-tyrosine	3.46	7.84	10.09	3.23	2.76
D-Leucyl-L-tyrosine	3.12	8.34	10.35	3.73	2.93

using Eq. (19). The calculated relaxation times are listed in the last column of Tables 9 and 10. The values of k_{-1} and k_{-2} were obtained from k_1/K_1 and k_2/K_2. All rate constants are compiled in Table 11.

Disscussion

Histidine anion has three binding sites. Imidazole sites coordinate at *trans*-configuration for ML_2 and MLD. These structures were solved with the X-ray intensity data by Frazer *et al.*[15]. As CD

Table 9 Experimental conditions and relaxation times for nickel (II)—L-leucyl-L-tyrosine solutions[a)]

$(Ni^{2+})_o$ (10^{-3}M)	$(L^-)_o$ (10^{-3}M)	pH	τ_{obs} (msec)	τ_{calc} (msec)
0.976	1.05	7.47	280	260
1.95	2.02	7.43	210	190
2.93	2.93	7.37	190	140
3.90	3.22	7.44	100	110
4.88	3.56	7.57	77	100
0.976	1.05	7.37	250	270
2.93	1.37	7.52	110	140
5.86	0.993	7.55	91	75
7.80	1.01	7.67	57	57
9.76	1.01	7.82	40	47

a) $(Ni^{2+})_o$ and $(L^-)_o$ represent the initial concentrations of nickel ion and dipeptide, respectively.
Phenol red was used as indicator (initial consentration $(In^-)_o = 1.30 \times 10^{-5}$M).
Hydrogen ion concentrations were calculated from pH values using activity coefficient of 0.80.
τ_{obs} and τ_{calc} represent the observed and calculated relaxation times, respectvely.

Table 10 Experimental conditions and relaxation times for nickel (II)—D-leucyl-L-tyrosine solutions[a)]

$(Ni^{2+})_o$ (10^{-3}M)	$(L^-)_o$ (10^{-3}M)	pH	τ_{obs} (msec)	τ_{calc} (msec)
4.08	1.09	7.87	55	106
2.04	1.18	7.63	880	184
6.12	1.14	7.78	91	74
2.04	3.78	7.45	210	180
10.20	1.39	7.87	38	47
4.08	4.03	7.68	93	110
8.16	4.28	7.59	49	55

a) Pdenol red was used as indicator (initial concentration $(In^-)_o = 1.39 \times 10^{-5}$M).

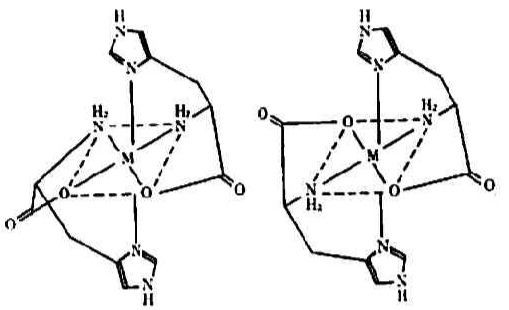
Table 11 Rate constants of complexation reactions of nickel (II) (25°C, ionic strength 0.15)

	k_1 ($M^{-1}sec^{-1}$)	k_{-1} (sec^{-1})	k_2 ($M^{-1}sec^{-1}$)	k_{-2} (sec^{-1})
L-Leucyl-L-tyrosine	2.2×10^3	1.3	1.7×10^3	3.0
D-Leucyl-L-tyrosine	2.4×10^3	0.45	2.0×10^3	2.4

spectra of the crystal and aqueous solution of the complex were identical, the structure of the complex

15) K. A. Fraser, H. A. Long, R. Candlin and M. M. Harding, *Chem. Comm.*, 1965, 344

in aqueous solution was considered to be identical with the crystalline state[16]. MLD is more stable than ML_2 or MD_2 due to the advantageous configuration in the interaction energy caused by the electrostatic interaction and/or the steric hindrance. Generally, the complex formation proceeds with the water liberation from the inner hydration shell of the metal ion through diffusion-controlled ion-pair formation[17]. If the ligand is multidentate, the more coordinated water molecules are released and the chelate rings are formed.



ML_2 MLD

The reaction between ML^+ and L^- may be shown as the following scheme (the rate constants of the individual steps are described as k_{01}, *etc.*):

$$\underset{\text{(I)}}{LM^+ + L^-} \overset{\text{fast}}{\rightleftarrows} \underset{\text{(II)}}{LM^+(H_2O)L^-} \underset{k_{-01}}{\overset{k_{01}}{\rightleftarrows}} \underset{\text{(III)}}{LM-L} \underset{k_{-02}}{\overset{k_{02}}{\rightleftarrows}} \underset{\text{(IV)}}{LM=L} \underset{k_{-03}}{\overset{k_{03}}{\rightleftarrows}} \underset{\text{(V)}}{LM\equiv L}. \qquad (22)$$

(II) is the ion-pair state and (III), (IV) and (V) are unidentate, bidentate and terdentate complex, respectively. The rate constants of other amino acid complex formation reactions of the type,

Table 12 Rate constants of complexation reactions of the type[19,20]

$$ML_{i-1} + L \xrightarrow{\vec{k}} ML_i$$

Metal ion	Ligand	$\vec{k}$ ($M^{-1}\,sec^{-1}$)
Ni (glycine)	glycine	5.6×10^4
Ni $(glycine)_2$	glycine	4.3×10^4
Ni (glycyl-glycine)	glycyl-glycine	9.2×10^3
Ni (α-alanine)	α-alanine	4.0×10^4
Co (glycine)	glycine	2.0×10^6
Co $(glycine)_2$	glycine	8.0×10^5
Co (glycyl-glycine)	glycyl-glycine	1.1×10^5
Co (α-alanine)	α-alanine	8.0×10^5

16) R. D. Gillard and S. H. Laurie, *J. Chem. Soc.*, (A) **1970**, 59
17) M. Eigen, *Z. Elektrochem.*, **64**, 115 (1969)
18) K. Kustin, R. F. Pasternack and E. M. Weinstock, *J. Am. Chem. Soc.*, **88**, 4610 (1966)
19) G. Davies, K. Kustin and R. F. Pasternack, *Inorg. Chem.*, **8**, 1535 (1969)
20) R. Fuoss, *J. Am. Chem. Soc.*, **80**, 5059 (1958)

$$ML_{i-1}+L \underset{}{\overset{\vec{k}}{\rightleftarrows}} ML_i \qquad (M=Ni^{2+}, Co^{2+}),$$

are shown in Table 12. The rate-determining step of complex formation reactions in Table 12 is the initial monodentate complex formation step.

If the closure of the chelate ring is the rate-determining step, the formation rate is retarded as seen in Co (II)-β-alanine system.

As the values of k_2 (or k_3) in Ni (II), Co (II)-histidine systems are comparable with those of Table 12, the rate-determining step may be monodentate complex formation step (II)-(III). If this mechanism is allowed, k_2 can be written as the product of an ion-pair formation constant K_a and k_{01}, identical with the rate constant for the inner sphere exchange of H_2O.

$$k_2=K_a k_{01}. \tag{23}$$

K_a is calculated from Fuoss's equation[20]:

$$K_a=\frac{4\pi Na^3}{3000}e^{-U(a)/kT}, \tag{24}$$

$$U(a)=\frac{z_1 z_2 N\varepsilon^2}{aD(1+\kappa a)}, \qquad \kappa=\frac{8\pi N\varepsilon^2}{1000DkT}\mu,$$

where μ is the ionic strength, N is Avogadro's number, a is the distance of closest approach of the ion-pair partners, z_i is the charge on the i-th ion, ε is the electric charge, k is the Boltzmann constant, and D is the dielectric constant of the solvent. The value of a is assumed to be 5Å[11]. K_a is calculated to be 0.80. The value of k_{01} is estimated from Eq. (21) using the values of k_2 and K_a; $5.1\times10^5\,sec^{-1}$ for NiL^+, $1.8\times10^6\,sec^{-1}$ for CoL^+, $7.5\times10^7\,sec^{-1}$ for ZnL^+, $9\times10^7\,sec^{-1}$ for CdL^+.

The value of k_{01} corresponds to the rate constant for the innersphere exchange of H_2O. For Ni^{2+} and Co^{2+}, the exchange rate constants are known by NMR, to be $2.7\times10^4\,sec^{-1}$ and $1.1\times10^6\,sec^{-1}$, respectively[21].

The dissociation products of MLD are $(ML^+ + D^-)$ or $(MD^+ + L^-)$, but those of ML_2 are $(ML^+ + L^-)$. To compare k_{-2} and k_{-3}, it is necessary to multiply the statistical factor 2 for k_{-3}. If there is no stereoselectivity, $2k_{-3}=k_{-2}$ is held. It is apparent from Table 7 that k_{-2} is about twice as much as $2k_{-3}$ for Ni (II), Co (II) and Zn (II). These relations correspond to the stereoselectivity in equilibrium constants.

The effect of the optical activity of histidine kinetically appears in the dissociation reaction of bis histidine complexes. These relations are schematically shown in Fig. 10 using the potential diagram.

The states (a) and (c) correspond to (I) and (V) in Eq. (22), and the state (b) is probably the five coordinated state of the metal ion in the ion-pair.

Recently, Barnes and Pettit[22] studied Ni (II), Zn (II)-histidine systems by calorimetry. The result is that the difference of the stability of ML_2 and MLD is caused by enthalpy terms, not by

21) T. J. Swift and R. E. Connick, *J. Chem. Phys.*, 37, 307 (1962); 41, 2553 (1964)

22) D. S. Barnes and J. P. Pettit, *J. Inorg. Nucl. Chem.*, 33, 2177 (1971)

entropy terms.

Therefore, the difference of dissociation rate constants of ML_2 and MLD is probably caused by activation enthalpy terms of the reaction.

Ni(II)-L-leucyl-L-tyrosine and Ni(II)-D-leucyl-L-tyrosine systems show the same behavior as Ni(II)-, Co(II)- and Zn(II)-histidine systems.

The values of k_1 for both systems are the same within experimental error, but the value of k_{-1} for Ni(II)-L-leucyl-L-tyrosine is about three times as much as that of Ni(II)-D-leucyl-L-tyrosine system from Table 11. The effect of the optical activity of these dipeptides is found kinetically in dissociation reactions. Li *et al.*[9] considered the following folded-unfolded model on the dissociation of the dipeptide.

$$\underset{\text{(a) unfolded}}{H_3^+NCH(R_1)CONHCH(R_2)COOH} \rightleftharpoons \underset{\text{(b) folded}}{H_3^+NCH(R_1)CONHCH(R_2)COO^-} \rightleftharpoons \underset{\text{(c) unfolded}}{H_2NCH(R_1)CONHCH(R_2)COO^-}.$$

The DL-isomer shows the R groups in a relative *trans* position to each other in the folded form. But the LL-isomer shows the R groups in a relative *cis* position and is more unstable in the folded form. Complexation with metal ions coincides with the transition of the dipeptides from the unfolded form (c) to the folded form (b).

The stability of the 1:1 complexes of these diastereoisomeric leucyltyrosine is different because the DL-isomer complex is more stable than the LL-isomer complex, if the R groups are large enough to inhibit facile complexation with these metal ions. The rate-determining step of the complex formation reaction may be the first unidentate complex formation step as shown in Fig. 10.

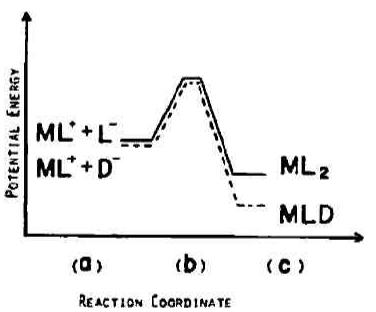


Fig. 10 Potential diagram for the complexation reactions of ML_2 and MLD

The value of k_1 in Ni(II)-leucine system is $1.7\times10^4 M^{-1}sec^{-1}$, which is larger than that of Ni(II)-leucyltyrosine system. This is due to the diminished accessibility of ion-pairing sites on leucyltyrosine. The simillar effect is observed in amino acid homologues[23,24].

Although the effect of the ligand nature on complexation reactions with metal ions is not well understood, several points are known to certain extent mainly in amino acids.

23) A. Kowalack, K. Kustin, R. F. Pasternack and S. Petrucci, *J. Am. Chem. Soc.* **89**, 3126 (1967)

24) J. Osugi and H. Nakatani, *Nippon Kagaku Kaishi*, (*J. Chem. Soc. Japan, Pure Chem. Sect.*), **1972**, 405

1. Chelate ring number: β-Amino acids form a six-membered chelate ring with the metal ion. The rate of complex formation reactions is retarded owing to the difficulty of the chelate formation. Such an effect was observed in β-alanine and β-amino butylic acid complexes[18,25].

2. Ligand charge: $\log k_1$ is in proportion to $\log K_a$ by Eq. (23) because k_{01} is in characteristic of the metal ion. As K_a relates with the charge product z_1z_2 from Fuoss's Eq. (24). The plot of $\log k_1$ *vs.* z_1z_2 should be a straight line. This plot was given in the complexation reaction of Ni^{2+} with various ligands by Cassat and Wilkins[14].

3. Ionic radius: It is desirable to use amino acid homologues for the study of the ionic radius effect because only the number of carbon chains may be effective to the complexation reactions. The effect was investigated by Kustin *et al.* on glycine homologues[23], and by Osugi *et al.*[24] on asparagine homologues. The rate of complexation reactions decreases with the increase of carbon chains due to the diminished accessibility of ion-pairing sites on the ligand.

4. Optical activity: As was clarified in this paper, when L- and DL-histidine or diastereoisomeric leucyltyrosine were used as ligands, there was no effect of the optical activity on the complex formation reaction rates, and the effect was found only in the dissociation reactions.

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