and J_{dipole}, or if B is a property of the Fermi contact term. The results suggest that the equation may be quite general for indirect coupling. For example, the work of Maher and Evans suggests that this relationship holds for aryl as well as alkyl and alkenyl systems. One must be careful to keep the molecular systems very similar in order to obtain good predictions.

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Thermodynamics and Kinetics of Complex Formation between Cobalt(II), Nickel(II), and Copper(II) with Glycyl-L-leucine and L-Leucylglycine

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Abstract: The thermodynamics of complex formation between cobalt(II), nickel(II), and copper(II) with glycyl-L-leucine and L-leucylglycine have been studied potentiometrically. Although the acidity constants of these two peptides and those of glycylglycine are nearly the same, the metal complexes of leucylglycine are considerably less stable than those of the glycyl dipeptides. The influence of the isobutyl group on the acidity constant for the deprotonation of the CuL^+ complex is also very dependent on the place of attachment. The CuL^+ complex of leucylglycine is far more acidic than those of the glycyl dipeptides. The kinetics of these reactions were studied using the temperature-jump technique. The forward rate constants for complexation show very little variation with the biggest effect appearing for the copper system. Even the rates of proton attack of the Cu(L–1H) species vary by less than a factor of 3 for the dipeptides. For the deprotonation effect, the rate constants correlate with the acidities of the CuL^+ species leading to a variation of about 100. The thermodynamic and kinetic results are consistent with the Rabin model (coordination *via* the amino end group and the oxygen of the amide group) for bonding in metal–dipeptide complexes.

Investigations of the thermodynamics of complex formation between metal ions of the 3d transition series and dipeptides have demonstrated that the stability of complexes formed with glycyl dipeptides is mainly determined by the basicity of the amino end group.[2,3] Other studies, focused on the kinetics, showed that zwitterions of oligopeptides are extremely unreactive in complex formation. The reactive species are the anionic forms of the peptides, *i.e.*, species with an unprotonated amino end group.[4–6]

X-Ray studies of solid metal ion–peptide complexes disclosed that the metal ion is bound to the amino end group and the oxygen of the neighboring amide group in the pH region in which the amide NH is not deprotonated.[7,8] Evidence for the same binding mode in aqueous solution was given by studies of the thermodynamics[2,9] and kinetics.[10] In such peptide complexes, especially with Cu^{2+} as the metal ion, the amide group may become deprotonated as the pH is increased and then the metal ion coordinates the amino end group and the nitrogen of the neighboring amide group.[7–10]

The aim of the present study is to learn how the rate of complex formation is influenced by the presence of a large alkyl group substituted at an α-carbon, *i.e.*, to see if steric hindrance is important. We used as ligands, glycyl-L-leucine or L-leucylglycine, and as metal ions, Co^{2+}, Ni^{2+}, or Cu^{2+}. Even though several of the corresponding stability constants of the complexes are already known it seemed justified to determine them all under a uniform set of conditions.

Experimental Section

A. Thermodynamics Experiments. Materials and Measurements. The metal perchlorates, glycyl-L-leucine, and L-leucylglycine were purchased from Fluka AG, Buchs, Switzerland. The measurements were performed by potentiometric titrations as previously described.[11]

Acidity Constants of the Dipeptides.[12] The values of $K^{H}_{H_2L}$ of the dipeptides were determined by titrating 10 ml of aqueous 4.4×10^{-2} M $HClO_4$ and $NaClO_4$ ($\mu = 0.1$) in the presence and absence of the ligands (3×10^{-2} M) under N_2 with 0.5 M NaOH and those of K^{H}_{HL} were determined by titrating 50 ml of aqueous 10^{-4} M $HClO_4$

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(2) (a) L. G. Sillen and A. E. Martell, *Chem. Soc. Spec. Publ.*, **No. 17**, (1964); (b) B. R. Rabin, *Trans. Faraday Soc.*, **52**, 1130 (1956).
(3) (a) S. P. Datta, R. Leberman, and B. R. Rabin, *ibid.*, **55**, 1982 (1959); (b) *ibid.*, **55**, 2141 (1959); (c) S. P. Datta and B. R. Rabin, *ibid.*, **52**, 1123 (1956).
(4) J. C. Cassatt, Ph.D. Thesis, State University of New York at Buffalo, 1969; J. C. Cassatt and R. G. Wilkins, *J. Amer. Chem. Soc.*, **90**, 6045 (1968).
(5) K. Kustin and R. F. Pasternack, *J. Phys. Chem.*, **73**, 1 (1969).
(6) G. Davies, K. Kustin, and R. F. Pasternack, *Inorg. Chem.*, **8**, 1535 (1969).
(7) H. C. Freeman in "The Biochemistry of Copper," J. Peisach, P. Aisen, and W. E. Blumberg, Ed., Academic Press, New York, N. Y., 1966, p 77 ff.
(8) H. C. Freeman, *Advan. Protein Chem.*, **22**, 257 (1967).
(9) H. Sigel, R. Griesser, and B. Prijs, *Z. Naturforsch. B*, **27**, 353 (1972).
(10) R. F. Pasternack, M. Angwin, and E. Gibbs, *J. Amer. Chem. Soc.*, **92**, 5878 (1970).
(11) R. Griesser and H. Sigel, *Inorg. Chem.*, **9**, 1238 (1970).
(12) Abbreviations used: M, general metal ion; L, general ligand; the symbol HL represents the zwitterionic form of the dipeptide.

with and without ligand (8×10^{-4} or 1.6×10^{-3} M) using 0.1 M NaOH (μ = 0.1; temperature 25°). The constants were calculated from the relation between pH and neutralization degree (between 10 and 90%). $K^{H}_{H_2L}$ and K^{H}_{HL} were evaluated from 4 and 27 independent titrations, respectively.

Stability Constants of the Co^{2+} and Ni^{2+} Complexes. The conditions of measurements for the determination of the stability constants, K^{M}_{ML}, were the same as for the acidity constants (volume of the reaction solution: 50 ml), but a part of $NaClO_4$ was replaced by $M(ClO_4)_2$ with the ratio M:HL = 7.5:1 or 15:1. Titrations of solutions without ligand were used as a basis for the evaluation. Each system was titrated at least five times. The calculation of K^{M}_{ML} was done by taking into account the species H_2L^+, HL, L^-, M^{2+}, ML^+, and ML_2.[11]

Previously, $K^{ML}_{ML_2}$ was determined from titrations where HL was in excess with regard to Co^{2+} or Ni^{2+}: $[M^{2+}] = 5.4 \times 10^{-4}$ or 8×10^{-4} M; the ratio M:HL was between 1:5 and 1:50; $[HClO_4] = 10^{-4}$ M (μ = 0.1); the volume of the reaction solution was 50 ml. With each metal ion at least nine independent titrations were carried out. The evaluation of the titration data was done according to Irving and Rossotti[13] by plotting $\bar{n}/(1 - \bar{n})[L]$ *vs.* $(2 - \bar{n})[L]/(1 - \bar{n})$. Only the values between $\bar{n}$ = 0.1 to 0.95 and 1.05 to 1.25 were used. The straight line was drawn using a regression method. The values with $\bar{n} > 1.25$ deviated somewhat from the straight lines and were therefore not taken into account. This may be due to the formation of 1:3 complexes, but more probably to a deprotonation of an amide group at higher pH values.[14]

Constants of the Cu^{2+} Systems. The conditions of measurements for the determination of the constants K^{Cu}_{CuL} and K^{H}_{CuL} were the same as for the acidity constants (volume of the reaction solution: 50 ml), but a part of $NaClO_4$ was replaced by $Cu(ClO_4)_2$ with the ratio Cu^{2+}:L = 1:1. Titrations of solutions without ligand (and Cu^{2+}) were used as a basis for the evaluation.[9] At least seven titrations were carried out for each system. In the case of the L-leucylglycine 1:1 system the species CuL^+ occurred only in insignificant amounts. Therefore, 15 additional titrations were made with a ratio Cu:L between 5:1 and 40:1.

B. Kinetics Experiments. Materials. The dipeptides used in this study were obtained from Nutritional Biochemicals Corp. Baker reagent grade nitrate salts of potassium(I), cobalt(II), nickel(II), and copper(II) were used without further purification. Stock solutions of the transition metal ions were prepared and the concentrations were determined *via* EDTA titrations using murexide as indicator.

Measurements. Solutions for kinetic runs were freshly prepared from the solid dipeptide and stock solutions of KNO_3, transition metal ion nitrate, and appropriate indicator. The solutions were degassed and maintained under a nitrogen atmosphere. The pH was adjusted by the dropwise addition of dilute NaOH and/or HNO_3 to ±0.01 pH unit. The solutions were equilibrated at 17° and the kinetics investigated by causing a sudden 8° temperature ump using an apparatus described elsewhere.[15]

Test solutions of either metal ion or ligand in the absence of the other showed no discernible effects within the time range of this instrument (20 μsec–200 msec). The relaxation times for solutions containing all the reactants represent an average of at least three photographic determinations. The relative error for solutions showing a single relaxation effect is ±10%. However, the copper systems at relatively high pH showed two relaxation effects; the relative error in relaxation times for these solutions is somewhat greater, that is, about ±25%.

Results

Thermodynamics. The constants of the following equilibria were determined by potentiometric titrations (μ = 0.1, $NaClO_4$; 25°).

$$H_2L^+ \rightleftharpoons HL + H^+ \qquad K^{H}_{H_2L} = [HL][H]/[H_2L] \quad (1)$$

$$HL \rightleftharpoons L^- + H^+ \qquad K^{H}_{HL} = [L][H]/[HL] \quad (2)$$

$$M^{2+} + L^- \rightleftharpoons ML^+ \qquad K^{M}_{ML} = [ML]/[M][L] \quad (3)$$

$$ML^+ + L^- \rightleftharpoons ML_2 \qquad K^{ML}_{ML_2} = [ML_2]/[ML][L] \quad (4)$$

With Cu^{2+} as a metal ion, the complex formed according to equilibrium 3 may be deprotonated at the amide group at relatively low pH values. Hence, in this case equilibrium 5 has additionally to be taken into account.

$$CuL^+ \rightleftharpoons Cu(L\text{–}1H) + H^+$$

$$K^{H}_{CuL} = [Cu(L\text{–}1H)][H]/[CuL] \quad (5)$$

Because of equilibrium 5, *i.e.*, the formation of Cu(L–1H), it is more convenient to calculate primarily the constants for equilibria 6 and 7.

$$Cu^{2+} + HL \rightleftharpoons CuL^+ + H^+$$

$$K^{H}_{(Cu+HL)} = [CuL][H]/[Cu][HL] \quad (6)$$

$$Cu^{2+} + HL \rightleftharpoons Cu(L\text{–}1H) + 2H^+$$

$$K^{2H}_{(Cu+HL)} = [Cu(L\text{–}1H)][H]^2/[Cu][HL] \quad (7)$$

The connection between eq 3 and 5 with 6 and 7 is given by the eq 8 and 9.

$$\log K^{Cu}_{CuL} = pK^{H}_{H_2L} + pK^{H}_{HL} - pK^{H}_{(Cu+HL)} \quad (8)$$

$$pK^{H}_{CuL} = pK^{2H}_{(Cu+HL)} - pK^{H}_{(Cu+HL)} \quad (9)$$

The determination of the constants due to equilibria 6 and 7 for the Cu^{2+}–glycyl-L-leucine system was achieved without difficulties. The results are $pK^{H}_{(Cu+HL)}$ = 5.52 ± 0.05 and $pK^{2H}_{(Cu+HL)}$ = 10.28 ± 0.06. However, in the Cu^{2+}–L-leucylglycine system the concentration of the species CuL^+ is always very low. This means the amino and the amide protons are set free in practically one step according to equilibrium 7. As a result the concentration of Cu(L–1H) is always in excess of the concentration of CuL^+. Even in the experiments with large excess of Cu^{2+} (*cf.* Experimental Section) the concentration of CuL^+ rises only to about 5% of the total Cu^{2+} present. Therefore, the experimental error in the constant of equilibrium 6 is rather large, while that in the constant of equilibrium 7 is much smaller: $pK^{H}_{(Cu+HL)}$ = 6.4 ± 0.2 and $pK^{2H}_{(Cu+HL)}$ = 9.66 ± 0.05.

In Figure 1 a representative set of experimental data obtained from potentiometric titrations is shown. The pH of the reaction solution is plotted against the neutralization degree. The formation of complexes shifts the buffer region of the ligand to lower pH values. Thus, from Figure 1 for the stability of the complexes the series follows, $Co^{2+} < Ni^{2+} < Cu^{2+}$, which is in accord with the Irving–Williams sequence.[16] But more important, it can be concluded that the Co^{2+} and Ni^{2+} complexes of glycyl-L-leucine are significantly more stable than the corresponding complexes of L-leucylglycine. A superficial glance at the curves due to the Cu^{2+} systems in Figure 1 suggests the opposite order of stability. However, in this case the situation is more complicated, as the amide groups are deprotonated. Therefore, it is not possible to draw an unequivocal conclusion without calculations.

In addition to the experimental data (curves) for the Cu^{2+} systems in Figure 1, a "redrawing" (points) of the titration curves is shown. This calculation was carried out with the *average result of all experiments.* Of course, if the results of the specific experimental curves shown in Figure 1 would have been taken, there would be no observable difference between the experimental and calculated curves.[9]

(13) H. Irving and H. S. Rossotti, *J. Chem. Soc.*, 3397 (1953).
(14) P. J. Morris and R. B. Martin, *Inorg. Chem.*, 10, 964 (1971).
(15) R. F. Pasternack, K. Kustin, L. A. Hughes, and E. Gibbs, *J. Amer. Chem. Soc.*, **91**, 4401 (1969).
(16) H. Irving and R. J. P. Williams, *J. Chem. Soc.*, 3192 (1953).

Table I. Thermodynamic Constants for Glycyl-L-leucine and L-Leucylglycine and Their Complexes with Co^{2+}, Ni^{2+}, or Cu^{2+} [a]

Dipeptide	$pK^{H}_{H_2L}$	pK^{H}_{HL}	Log K^{Co}_{CoL}	Log $K^{CoL}_{CoL_2}$	Log K^{Ni}_{NiL}	Log $K^{NiL}_{NiL_2}$	Log K^{Cu}_{CuL}	pK^{H}_{CuL}
Glycylglycine	3.20[b]	8.23[b]	3.23[b]	2.56[b]	4.49[c]	3.42[c]	5.82[d]	4.25[d]
	3.21[e]	8.13[f]					5.71[f]	4.15[f]
Glycyl-L-leucine[g]	3.13 ± 0.01	8.28 ± 0.01	3.25 ± 0.01	2.77 ± 0.18	4.25 ± 0.02	3.74 ± 0.08	5.89 ± 0.05	4.76 ± 0.06
L-Leucylglycine[g]	3.05 ± 0.01	8.10 ± 0.01	2.42 ± 0.02	2.0 ± 0.4	3.44 ± 0.02	2.99 ± 0.15	4.8 ± 0.2[h]	3.3 ± 0.2[h]

[a] Indicators used in this study are Bromothymol Blue, $pK^{H}_{Hln} = 7.10$, and Methyl Red, $pK^{H}_{Hln} = 5.00$: I. M. Kolthoff, *J. Phys. Chem.*, **34**, 1466 (1930). [b] 25°; S. P. Datta and B. R. Rabin, *Trans. Faraday Soc.*, **52**, 1117 (1956). [c] 25°: N. C. Li, E. Doody, and J. M. White, *J. Amer. Chem. Soc.*, **79**, 5859 (1957). [d] 25°; ref 3c. [e] M. K. Kim and A. E. Martell, *J. Amer. Chem. Soc.*, **85**, 3080 (1963). [f] $\mu = 0.1$; 25°; ref 9. [g] The uncertainties given are three times the standard deviation. [h] *Cf.* text.

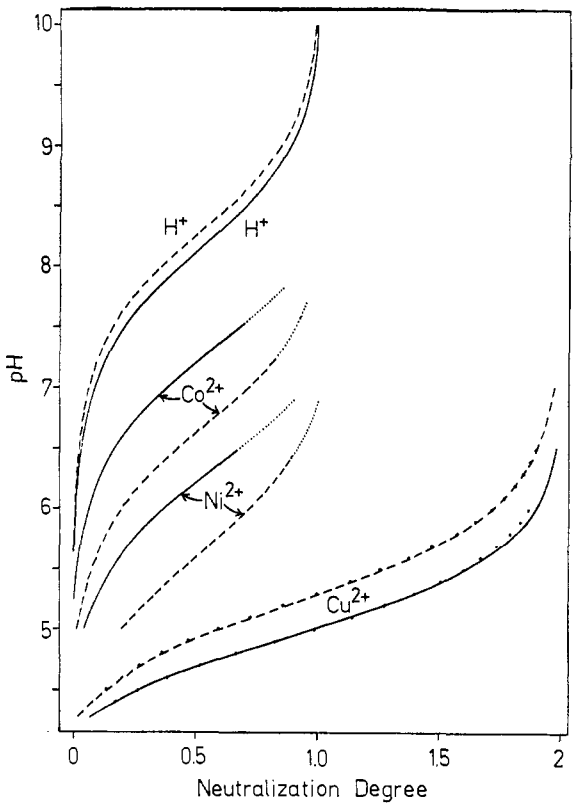


Figure 1. Dependence of neutralization degree upon pH during potentiometric titration of L-leucylglycine (full lines) or glycyl-L-leucine (broken lines) with and without metal ions in aqueous solution ($I = 0.1$, $NaClO_4$; 25°). The dotted-line portions extended toward higher degrees of neutralization indicate uncertainty due to hydrolysis. The dipeptide was 8×10^{-4} *M*, and Cu^{2+} when present was also 8×10^{-4} *M*. In the case of Co^{2+} and Ni^{2+} the dipeptide was 1.6×10^{-3} *M* and the metal ion 2.4×10^{-2} *M*. The points shown with the Cu^{2+} curves are for comparison; they were calculated with the average results of all titrations.

In Table I the equilibrium constants determined for the glycyl-L-leucine and L-leucylglycine systems with Co^{2+}, Ni^{2+}, or Cu^{2+} are summarized. For comparison, the corresponding data taken from the literature for the glycyl-glycine system are also given. The agreement between the present results and the data available in the literature[1] is good, with the exception of the Cu^{2+}–leucylglycine system. For the latter we computed a value of 4.8 for log K^{Cu}_{CuL}, while Rabin, *et al.*,[3b] found 5.3. For pK^{H}_{CuL} the values are 3.3 and 3.8, respectively. It is interesting to compare the constants due to equilibria 6 and 7. Using our acidity constants and the given results of Rabin, *et al.*,[3b] one calculates $pK^{H}_{(Cu+HL)} = 5.85$ (eq 8) and $pK^{2H}_{(Cu+HL)} = 9.65$ (eq 9). The latter value is in excellent agreement with our own while the first one is rather different. This indicates that the difference in the results is likely due to the above-mentioned low concentration of CuL^+. Additionally, Rabin, *et al.*, evaluated their data for K^{Cu}_{CuL} using the assumption, $[H^+] \gg K^{H}_{CuL}$, which is not satisfied for the Cu^{2+}–leucylglycine system.[3c] As we were able to redraw completely the experimentally obtained titration curve (*cf.* Figure 1) with our results, we believe that these newer values are more correct.

Kinetics. The reactions studied for nickel(II) and cobalt(II) are of the type

$$M^{2+} + L^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ML^+ \qquad ML^+ + L^- \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} ML_2 \tag{10}$$

The processes (10) are coupled to the reactions 1, 2, and

$$HIn \rightleftharpoons In^- + H^+ \tag{11}$$

Protolytic reactions of these types are very rapid[17] and can be assumed to reach equilibrium far more quickly than reactions involving metal-containing species. All the pertinent equilibrium constants used are those of Table I. There proved to be no need to consider polymeric structures in the kinetic analysis; where studies are available for these systems, polymeric structures have been ruled out both in the solid state[18] and in solution.[19] Furthermore, although there was considerable variation in hydrogen ion concentration, most often greater than a factor of 10, the rate constants showed no pH dependence. This result is consistent with other studies which have demonstrated that zwitterions of the oligopeptides are extremely unreactive.[4-6]

A general treatment for obtaining relaxation time expressions for reactions of type 10 has been developed.[20] The relaxation times for such systems are obtained from

$$\tau^{-2} - (a_{11} + a_{22})\tau^{-1} + (a_{11}a_{22} - a_{12}a_{21}) = 0 \tag{12}$$

where

$$a_{11} = k_1\left(\frac{[M]}{1+\alpha} + [L] + 1/K^{M}_{ML}\right) \qquad a_{12} = k_1\left(\frac{1}{K^{M}_{ML}} - \frac{[M]}{1+\alpha}\right)$$
$$a_{21} = k_2\left([L] - \frac{[ML]}{1+\alpha}\right) \qquad a_{22} = k_2\left(\frac{[ML]}{1+\alpha} + [L] + 1/K^{ML}_{ML_2}\right) \tag{13}$$

(17) M. Eigen, W. Kruse, G. Maass, and L. DeMaeyer, *Progr. React. Kinet.*, **2**, 286 (1964).
(18) M. L. Bair and E. M. Larsen, *J. Amer. Chem. Soc.*, **93**, 1140 (1971).
(19) J. F. Boas, J. R. Pilbrow, C. R. Hartzell, and T. D. Smith, *J. Chem. Soc. A*, 3159 (1969).
(20) G. G. Hammes and J. I. Steinfeld, *J. Amer. Chem. Soc.*, **84**, 4639 (1962).

and

$$\alpha = \frac{K^{H}_{H_2L}[H^+] + \beta[H^+][HL] + [H^+]^2 + \beta K^{H}_{H_2L}[OH^-] + \beta[H^+][OH^-]}{K^{H}_{HL}K^{H}_{H_2L} + 4\beta K^{H}_{HL}[HL] + \beta K^{H}_{H_2L}[L^-] + \beta K^{H}_{HL}K^{H}_{H_2L}[OH^-]/[H^+]}$$

and

$$\beta = \frac{K^{H}_{In} + [H^+]}{K^{H}_{In} + [H^+] + [In^-]} \qquad (14)$$

The experimental conditions and a summary of the observed relaxation times are shown in Tables II–V.

Table II. Relaxation Experiments for Cobalt(II)–Glycylleucine Reactions (25°, μ = 0.1 M)[a]

10^3 [Co]$_0$	10^3 [glyleu]$_0$	pH	τ_{obsd}, msec	τ_{calcd}, msec
2.50	5.21	6.47	1.2	0.96
2.50	5.21	7.22	1.1	0.86
2.50	20.0	6.32	1.1	0.91
2.50	20.0	7.30	0.77	0.73
2.50	30.0	6.54	0.80	0.87
2.50	30.1	7.27	0.63	0.62
5.00	10.0	6.30	0.64	0.58
5.00	10.0	7.17	0.50	0.58
5.00	20.0	6.46	0.55	0.56
5.00	20.0	7.37	0.46	0.69
5.00	29.9	6.41	0.57	0.58
5.00	29.9	7.30	0.42	0.66
5.00	50.4	6.47	0.48	0.63
5.00	50.4	7.14	0.33	0.56
10.0	5.10	6.37	0.34	0.33
10.0	5.10	7.24	0.33	0.30
10.0	10.1	6.47	0.33	0.30
10.0	10.1	7.42	0.28	0.33

$k_1 = 3.5 \times 10^5\ M^{-1}\ sec^{-1}$ $k_2 = 3.3 \times 10^5\ M^{-1}\ sec^{-1}$
$k_{-1} = 2.0 \times 10^2\ sec^{-1}$ $k_{-2} = 5.6 \times 10^2\ sec^{-1}$

[a] All solutions are 5.0×10^{-5} M in Bromothymol Blue.

Table III. Relaxation Experiments for Cobalt(II)–Leucylglycine Reactions (25°, μ = 0.1 M)[a]

10^3 [Co]$_0$	10^3 [leugly]$_0$	pH	τ_{obsd}, msec	τ_{calcd}, msec
2.50	4.84	7.40	0.98	1.1
5.00	9.60	6.65	0.68	0.82
5.00	9.60	7.25	0.82	0.81
10.0	4.81	6.57	0.62	0.53
10.0	4.81	7.15	0.57	0.51
10.0	14.8	6.67	0.48	0.51
10.0	14.8	7.14	0.48	0.50
5.00	19.2	6.68	0.69	0.81
5.00	19.2	7.14	0.71	0.60
5.00	28.7	6.56	0.81	0.81
5.00	28.7	7.18	0.60	0.55
3.00	28.8	6.71	0.53	0.62
3.00	28.8	7.52	0.63	0.43
3.00	19.2	6.90	0.64	0.62
3.00	19.2	7.38	0.72	0.53
5.00	44.6	6.76	0.69	0.59
5.00	44.6	7.33	0.47	0.44

$k_1 = 1.4 \times 10^5\ M^{-1}\ sec^{-1}$ $k_2 = 1.4 \times 10^5\ M^{-1}\ sec^{-1}$
$k_{-1} = 5.3 \times 10^2\ sec^{-1}$ $k_{-2} = 1.4 \times 10^3\ sec^{-1}$

[a] All solutions are 4.9×10^{-5} M in Bromothymol Blue.

The calculated relaxation times are shown for comparison. Equation 12 was used in these calculations with trial values of the rate constants. The rate constants which best fit the data are given in Table VIII.

Table IV. Relaxation Experiments for Nickel(II)–Glycylleucine Reactions (25°, μ = 0.1 M)[a]

10^3 [Ni]$_0$	10^3 [glyleu]$_0$	pH	τ_{obsd}, msec	τ_{calcd}, msec
5.08	10.1	6.55	64	61
5.08	10.1	7.46	72	83
5.08	19.4	6.37	66	72
5.08	19.4	7.40	73	60
5.08	30.2	6.36	78	83
10.2	5.07	6.62	40	32
10.2	5.07	7.21	35	34
10.2	10.0	6.77	37	33
10.2	10.0	7.74	34	36
20.3	5.10	6.67	27	21
20.3	5.10	7.17	29	22
20.3	15.0	6.30	18	16
20.3	15.0	7.04	15	18

$k_1 = 3.5 \times 10^3\ M^{-1}\ sec^{-1}$ $k_2 = 8.6 \times 10^3\ M^{-1}\ sec^{-1}$
$k_{-1} = 0.20\ sec^{-1}$ $k_{-2} = 1.6\ sec^{-1}$

[a] All solutions are 5.0×10^{-5} M in Bromothymol Blue.

Table V. Relaxation Experiments for Nickel(II)–Leucylglycine Reactions (25°, μ = 0.1 M)[a]

10^3 [Ni]$_0$	10^3 [leugly]$_0$	pH	τ_{obsd}, msec	τ_{calcd}, msec
11.5	10.0	7.50	47	48
5.74	4.84	6.60	86	73
5.74	4.84	7.20	78	70
23.0	4.83	7.17	28	25
23.0	4.83	6.68	29	25
23.0	14.4	6.66	20	24
23.0	14.4	7.14	20	25
11.5	4.86	6.67	50	45
11.5	4.86	7.40	47	44
5.74	19.2	6.59	64	72
5.74	19.2	7.17	62	73
5.74	10.2	7.26	77	73
5.74	29.4	6.43	64	73
5.74	29.4	7.25	58	63
5.74	48.1	6.51	72	75
5.74	48.1	7.32	51	46

$k_1 = 2.0 \times 10^3\ M^{-1}\ sec^{-1}$ $k_2 = 2.4 \times 10^3\ M^{-1}\ sec^{-1}$
$k_{-1} = 0.73\ sec^{-1}$ $k_{-2} = 2.5\ sec^{-1}$

[a] All solutions are 4.9×10^{-5} M in Bromothymol Blue.

The situation for copper is somewhat different than that for nickel(II) and cobalt(II). The reactions to be considered are

$$Cu^{2+} + L^- \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} CuL^+ \underset{k_H}{\overset{k_D}{\rightleftharpoons}} Cu(L\text{-}1H) + H^- \qquad (15)$$

Under the conditions of these studies, it can be shown to be unnecessary to consider higher order complexes of Cu^{2+} with these dipeptides.[21] An analysis of this kinetic situation in which no assumption[10] is made about the relative rates of reactions 15 leads to the relaxation expression of the same form as (12) but now the a_{ij} are defined differently

$$a_{11} = k_1([Cu]/\alpha_1 + [L] + 1/K^{Cu}_{CuL})$$
$$a_{12} = k_1(1/K^{Cu}_{CuL} - [Cu]/\alpha_2)$$
$$a_{21} = k_H\left(K^{CuL}_{(CuL-1H)} - \frac{[Cu(L\text{-}1H)]}{\alpha_3}\right) \qquad (16)$$
$$a_{22} = k_H\left(\frac{Cu(L\text{-}1H)}{\alpha_4} + [H^+] + K^{CuL}_{Cu(L-1H)}\right)$$

(21) H. Dobbie and W. O. Kermack, *Biochem. J.*, **59**, 246 (1955).

where

$$\alpha_1 = N/D_1 \qquad \alpha_3 = N/D_3 \tag{17}$$
$$\alpha_2 = N/D_2 \qquad \alpha_4 = N/D_4$$

and

$$N = K^{H}_{H_2L}[L^-] + \omega K^{H}_{H_2L}K^{H}_{HL} + 4K^{H}_{HL}[HL] + \omega K^{H}_{H_2L}[H^+] + K^{H}_{H_2L}[H_2L^+] + [H^+]^2$$

and

$$\begin{aligned} D_1 &= \omega K^{H}_{H_2L}K^{H}_{HL} + K^{H}_{H_2L}[L^-] + 4K^{H}_{HL}[HL] \\ D_2 &= K^{H}_{H_2L}[L^-] + 2K^{H}_{HL}[HL] \\ D_3 &= 2[H^+]^2 + K^{H}_{H_2L}[H^+] \\ D_4 &= K^{H}_{H_2L}K^{H}_{HL} + K^{H}_{H_2L}[H^+] + [H^+]^2 \end{aligned} \tag{18}$$

and

$$\omega = \frac{K^{H}_{In} + [H^+] + [In^-]}{K^{H}_{In} + [H^+]} + \frac{[OH^-]}{[H^+]}$$

The experimental conditions and a summary of the observed and calculated relaxation times are shown in Tables VI–VII. As may be seen from these tables several of the copper solutions showed two relaxation times. For glycylleucine the second, slower effect appeared at relatively high pH where pH $\approx$ pK^{H}_{CuL}. Whereas all the effects are coupled, the faster effect depends primarily on the rate constants for complexation, k_1 and k_{-1}, whereas the relaxation time of the slower effect is particularly dependent on k_D and k_H. For the Cu^{2+}–leucylglycine system, no relaxation results could be obtained below the pK^{H}_{CuL} of CuL^+ which is 3.3. Related to this relatively small value of pK for this system is the fact that the concentration of CuL^+ is never appreciable in our experiments. Furthermore, the "second" effect which is observed for some solutions is faster than the "first" effect. We found that we could fit the data better for the assignment that τ_1 is primarily the protonation reaction and τ_2 the complexation reaction (*cf.* Table VII), although again all the reactions are coupled. This assignment is consistent with the thermodynamic results, provides a better fit of the kinetic data, and leads to rate constants which correlate with those of other closely related systems. A summary of the rate constants for the copper systems is given in Table VIII.

The fit between the calculated and observed relaxation times as shown in Tables VI and VII might be expected to be somewhat poorer for these copper dipeptide systems showing two relaxation effects since there is greater error in the determined relaxation times. However, the agreement is quite good for most of the solutions and about the same as that obtained for a somewhat simpler system to interpret (*i.e.*, no possibility of general acid catalysis or additional complexation steps to those considered) showing coupled relaxation effects.[22] Some solutions which appear rather similar in conditions differ in the number of effects observed. However, for systems as complicated as these, bicipital relaxation phenomena are occurring and minor changes in conditions can lead to large difference in the appearance and number of observable effects.[23]

Table VI. Relaxation Experiments for Copper(II)–Glycylleucine Reactions (25°, μ = 0.1 M)[a]

10^3 [Cu]$_0$	10^3 [glyleu]$_0$	pH	τ_1, msec Obsd	τ_1, msec Calcd	τ_2, msec Obsd	τ_2, msec Calcd
2.52	5.04	3.53	2.1	2.1		
2.52	5.04	4.00	1.1	1.5	8.0	6.6
2.52	20.1	3.47	2.0	1.9		
2.52	20.1	4.56	0.46	0.53	7.1	12
5.05	10.1	4.42	0.67	0.44	10	7.7
5.05	10.1	4.02	1.8	1.0		
5.05	20.0	3.77	1.7	1.4		
5.05	20.0	4.44	0.70	0.47	6.6	8.2
5.05	30.0	3.38	1.0	1.6		
5.05	30.0	4.18	0.81	0.74	5.0	6.5
10.1	5.07	3.77	1.3	1.2		
10.1	10.1	3.45	1.3	1.4		

$k_1 = 3.1 \times 10^8\ M^{-1}\ sec^{-1}$, $k_{-1} = 400\ sec^{-1}$; $k_D = 15\ sec^{-1}$, $k_H = 8.5 \times 10^5\ M^{-1}\ sec^{-1}$

[a] All solutions are 5.0×10^{-5} M in Methyl Orange.

Table VII. Relaxation Experiments for Copper(II)–Leucylglycine Reactions (25°, μ = 0.1 M)[a]

10^3 [Cu]$_0$	10^3 [leugly]$_0$	pH	τ_1, msec Obsd	τ_1, msec Calcd	τ_2, msec Obsd	τ_2, msec Calcd
2.80	29.5	4.20	2.7	2.7		
2.80	20.1	4.50	1.9	2.1		
2.80	20.1	3.59	2.2	2.2		
2.80	28.9	4.50	2.1	2.2		
2.80	28.9	3.58	1.7	2.1		
5.60	9.75	3.93	2.2	2.2		
5.60	20.4	4.48	1.4	1.3		
5.60	28.8	4.49	1.7	1.5	0.32	0.30
5.60	28.8	3.46	2.0	1.7		
5.60	47.9	4.49	1.6	1.7	0.26	0.29
5.60	47.9	3.61	1.7	1.9		
11.2	4.83	4.49	1.0	0.90	0.18	0.21
11.2	4.83	3.60	1.9	1.8		
11.2	10.3	4.49	0.90	0.87	0.29	0.22
11.2	9.70	4.42	1.5	0.79	0.24	0.25

$k_1 = 1.0 \times 10^8\ M^{-1}\ sec^{-1}$, $k_{-1} = 1.6 \times 10^3\ sec^{-1}$; $k_D = 1.2 \times 10^3\ sec^{-1}$, $k_H = 2.3 \times 10^6\ M^{-1}\ sec^{-1}$

[a] All solutions are 5.1×10^{-5} M in Methyl Orange.

Discussion

From the thermodynamic constants assembled in Table I several conclusions may be drawn. Since the acidity constants of the three dipeptides are nearly equal, the stability constants, K^{M}_{ML} and $K^{ML}_{ML_2}$, of their metal ion complexes may be directly compared. The conclusion based on Figure 1 that the Co^{2+} and Ni^{2+} complexes of glycyl-L-leucine are more stable than those of L-leucylglycine is confirmed by the calculated constants given in Table I, and the same order of stability holds for the Cu^{2+} complexes. The metal ion–glycylglycine and –glycyl-L-leucine complexes are practically of the same stability, while the L-leucylglycine complexes are significantly less stable. This can be explained by taking into account that presumably all three metal ions are bound in these complexes to the amino end group and the oxygen of the amide group. Hence, substitution at the carbon neighboring the amino end group, as in leucylglycine, results in considerable steric hindrance to complex formation, while in glycylleucine the isobutyl group two atoms distant from

(22) G. Davies, K. Kustin, and R. F. Pasternack, *Int. J. Chem. Kinet.*, 1, 45 (1969).

(23) R. W. Taylor and D. B. Rorabacher, *J. Phys. Chem.*, 76, 452 (1972).

Table VIII. Rate Constants for Reactions of Several Dipeptides with Nickel(II), Cobalt(II), and Copper(II)

	k_1, M^{-1} sec^{-1}	k_{-1}, sec^{-1}	k_2, M^{-1} sec^{-1}	k_{-2}, sec^{-1}
		Nickel(II)		
Glycylglycinate$^-$ [a]	3.2×10^3	0.27	9.2×10^3	4.2
Glycylsarcosinate$^-$ [b]	2.0×10^3	0.072	8.0×10^3	1.8
Glycyleucinate$^-$	3.5×10^3	0.20	8.6×10^3	1.6
Leucylglycinate$^-$	2.0×10^3	0.73	2.4×10^3	2.5
		Cobalt(II)		
Glycylglycinate$^-$ [a]	2.0×10^5	2.0×10^2	1.6×10^5	8.4×10^2
Glycylsarcosinate$^-$ [b]	4.6×10^5	0.75×10^2	8.0×10^5	3.0×10^2
Glycylleucinate$^-$	3.5×10^5	2.0×10^2	3.3×10^5	5.6×10^2
Leucylglycinate$^-$	1.4×10^5	5.3×10^2	1.4×10^5	14×10^2
	k_1, M^{-1} sec^{-1}	k_{-1}, sec^{-1}	k_D, sec^{-1}	k_H, M^{-1} sec^{-1}
		Copper(II)		
Glycylglycinate$^-$	3.5×10^8	9.6×10^2	67	8.7×10^5
Glycylsarcosinate$^-$ [c]	4.0×10^8	1.7×10^2		
Glycylleucinate$^-$	3.1×10^8	4.0×10^2	15	8.5×10^5
Leucylglycinate$^-$	1.0×10^8	16×10^2	1.2×10^3	2.3×10^6

[a] Reference 6. The values of k_{-1} and k_{-2} for Ni(glygly)$^+$ reported in ref 6 were in error. The correct values are shown above. [b] Reference 5. [c] Reference 10.

the coordinating oxygen of the amide group has little effect on coordination.

The influence of the isobutyl group on the acidity constants, K^H_{CuL}, of the Cu^{2+}–dipeptide complexes, CuL^+, is also very dependent on the place of substitution. The CuL^+ complex of glycylleucine is somewhat less acidic than that of glycylglycine. This, as the kinetic results imply, is probably the result of steric hindrance of the isobutyl group on the reorganization of the complex when the amide group is deprotonated and the binding site of the metal ion changes from the oxygen to the nitrogen of the amide group. Surprising is the high acidity of the Cu^{2+}–leucylglycine complex, CuL^+. The isobutyl substituent at the carbon neighboring the amino end group strongly favors the deprotonation of the amide group. This effect is unequivocal even though it is not easily explained; it manifests itself kinetically in an increase in the rate of deprotonation. This suggests that ring opening with the breaking of the Cu–O peptide bond may be rate determining and is facilitated by having a bulky group on the α-carbon atom.

It is interesting to compare, for the Cu^{2+}–dipeptide 1:1 mixtures, the concentration of the various species as a function of pH. These dependences are shown in Figure 2. In all three 1:1 mixtures the concentration of CuL^+ is rather low, especially with leucylglycine where the species CuL^+ reaches in the maximum only 1.2% of the total Cu^{2+} present.

This fact, that the concentration of CuL^+ is always very small in solutions containing Cu^{2+} and one of the mentioned dipeptides in a ratio 1:1, has its consequences on the interpretations and conclusions of the results given in a recent paper.[18] Bair and Larsen had prepared the solid complexes that had retained the peptide-amide proton, *i.e.*, ML^+, for Ni^{2+}, Cu^{2+}, and Zn^{2+} with glycylglycine, glycyl-L-leucine, or L-leucylglycine. These authors concluded: "The infrared spectra of the solid-state and visible spectra of the solutions are consistent with the bonding of the peptide-amide nitrogen in the copper complexes, and the peptide-amide oxygen in the nickel (zinc) complexes."[18] From our present results, we can comment only on the situation in solution, where we agree with their conclusions regarding the Ni^{2+} and Zn^{2+} complexes. However, from the conclusions to be drawn from Figure 2, *i.e.*, the low concentration of the species CuL^+ in all three systems, we cannot accept Bair and Larsen's conclusion regarding the binding sites in the copper complexes.

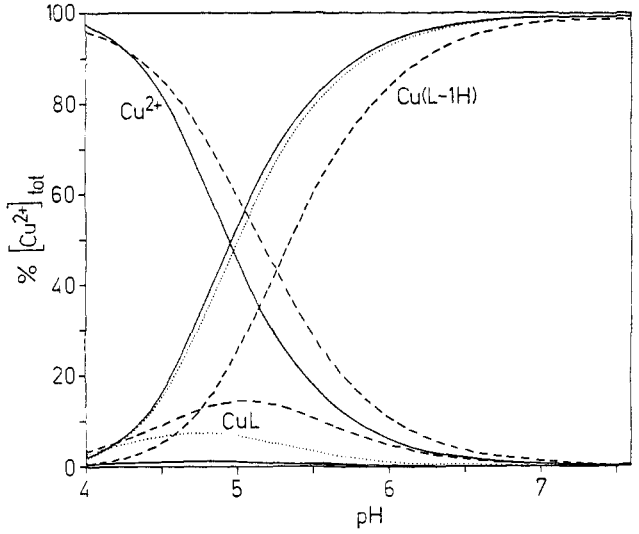


Figure 2. Influence of pH on the concentrations (given as the percentage of the total Cu^{2+} present) of the several species present in aqueous solution of Cu^{2+} and L-leucylglycine (full lines) or glycyl-L-leucine (broken lines). The computation made use of the constants of Table I and concentrations of 10^{-3} M for each reactant. Under the same conditions, the distribution of the species present in the Cu^{2+}–glycylglycine system[9] (dotted lines) is also shown for comparison.

The mentioned visible spectra[18] were measured "of aqueous solutions made by dissolving the solid in doubly distilled water. The concentrations are of the range 1×10^{-2} to 2.5×10^{-2} M for the copper(II) solutions. No adjustments were made in the pH, nor were any buffers added." For a 10^{-2} M solution of Cu(leucylglycine)Cl a pH of 4.6 was measured.[18] Taking these experimental conditions as a basis and using our results of Table I we calculate for the 1:1 mixtures at pH 4.6 and concentrations of 10^{-2} or 2.5×10^{-2} M for the reactants the following distribution of species (given as

the percentage of the total Cu^{2+} present): with glycylglycine CuL^+ is 17.1 or 20%, and Cu(L–1H) 48.4 or 56.4%; with glycyl-L-leucine CuL^+ is 32.3 or 40%, and Cu(L–1H) 22.3 or 27.8%; and with L-leucylglycine CuL^+ is 2.97 or 3.55% and Cu(L–1H) 57.9 or 69.1%. Hence, the ratio of [Cu(L–1H)]/[CuL] for the glycylglycine system is 2.8, for glycyl-L-leucine 0.7, and for L-leucylglycine 19.5. For the glycylglycine and glycylleucine systems these ratios are the lower limits, because the "natural" pH obtained in a solution by dissolving Cu(L)Cl must be higher (and therefore favor the formation of Cu(L–1H); *cf.* Figure 2) than by dissolving Cu(leucylglycine)Cl. The latter complex is the stronger acid as the data given in Table I demonstrate. Therefore, the solutions measured by Bair and Larsen at best contained CuL^+ and Cu(L–1H) in comparable amounts, but probably more of the latter species. In addition, the molar extinction coefficients, ϵ, are larger (by a factor of >2)[18,21,24] for Cu^{2+}–peptide complexes with deprotonated amide groups, compared with the undeprotonated complexes. Hence, the absorption maxima measured are due to the deprotonated complexes, Cu(L–1H), and *not* due to CuL^+ as concluded by the mentioned authors.[18,25] Of course, their conclusion from the spectra that two nitrogens are bound to the coordination square of Cu^{2+} is basically correct, but this conclusion applies to the deprotonated complexes and those are known to coordinate with the nitrogen of the amide group.[7–10] Therefore, the *experimental results* of Bair and Larsen are not in contradiction with other studies[9,10] that demonstrated binding of Cu^{2+} to the oxygen atom of the protonated amide group which is also in accord with the kinetic results.

The kinetics of complexation reactions of nickel(II), cobalt(II), and copper(II) have been studied quite extensively using a variety of rapid reaction techniques, but primarily stopped-flow and temperature-jump. The results of these studies have been tabulated and discussed elsewhere;[26,27] the conclusion drawn is that the substitution reactions of nickel(II) and cobalt(II) and perhaps copper(II)[28,29] proceed *via* a mechanism in which loss of a water molecule from the inner coordination sphere of a thermodynamically stable metal-containing species is rate determining. Whereas several examples have been found in which the rate is dependent on the nature of the incoming ligand,[27] these cases are fairly rare and depend on some readily identifiable property of the metal–ligand system as, for example, the formation of a six-membered[30–32] chelate ring. For most systems which have been studied, the rate constant for the formation of the monosubstituted complex, ML, depends on the identity of the metal ion, M, and on the charge type of the ligand; the rate constant is given by $k_1 = K_{a1}k_{01}$. In this expression K_{a1} is the equilibrium constant for ion-pair formation between $M(H_2O)_6^{2+}$ and L, and k_{01} is the rate constant for water loss from the inner coordination sphere of the fully aquated metal ion. Whereas K_{a1} is expected to show some variation due to structural features of the attacking ligand, this variation is usually very small and most often for ligands of a given charge within a factor of 2. Rather, the value of K_{a1} depends most strongly on the charge type of the reacting species.[33] Therefore, the most apparent and unusual feature of the complexation reactions involving dipeptides and oligopeptides is that the rate constants for formation of ML are generally about an order of magnitude smaller than those for the α-amino acids although the formal charge type of the reacting species is the same in both cases. This result has been interpreted as indicating that ion pairs in which the carboxylate end of the molecule are in proximity to the metal–water sheath do not lead to product, and only the fraction of the ion-pairs in which the amino end of the peptide is in proximity to the inner coordination sphere leads to reaction.[4,10] This has been taken as an indication that the carboxyl end of the molecule is not involved in the bonding of the ligand to the metal ion[34] which is in accord with the results of a thermodynamic study.[9] It is interesting to note that although this important kinetic difference exists for the peptides as compared to the amino acids, the straight line plots constructed by Rabin and coworkers[2,3] correlating log K^{M}_{ML} with pK^{H}_{HL} and log $K^{ML}_{ML_2}$ with pK^{H}_{HL} contain the amino acid as well as the respective dipeptides. In other words, the small values of k_1 (and k_2) for the dipeptides are balanced out by relatively small values for k_{-1} (and k_{-2}) so that the ratios correlate with those for the amino acids. If the suggestion relating the forward rate constant effect with the noninvolvement of the carboxyl group is correct, the reverse rate constant effect may be related to the solvating of this free carboxyl for the dipeptide as compared to the solvation of the bound carboxyl end of the molecule in the amino acid. In other words, the transition state complex may be stabilized for the amino acid case because of the increased solvation of the now-free carboxylate group whereas no such additional stabilization would occur for the dissociation of the metal–peptide complex.

When considering the results for the dipeptides alone we see that for each of the metal ions there is little variation in the values of k_1. For nickel(II), a normal range of $k_1 = (2\text{–}4) \times 10^3\ M^{-1}\ sec^{-1}$ and for cobalt(II), a range of $k_1 = (2\text{–}5) \times 10^5\ M^{-1}\ sec^{-1}$ can be established and the presence of nonpolar side groups on either portion of the dipeptide has little effect on rate constant. Differences in stabilities on the ML species arise from differences in the dissociation rate constants; the variation here is an order of magnitude. The values of k_2 also show little variation although the rate constants

(24) H. Sigel and G. Blauer, *Helv. Chim. Acta*, **51**, 1246 (1968).

(25) One may note at this point that the absorption maximum measured[18] for the Cu^{2+}–leucylglycine system (641 nm) is somewhat lower (and nearly identical with the maximum given for Cu(L–1H) of Cu^{2+}–glycylglycine; 638 nm) than the maxima of the corresponding glycylglycine and glycylleucine systems (649 nm). This is in accord with our experimental conclusion that practically all complexed Cu^{2+} in a 1:1 leucylglycine system is present as Cu(L–1H).

(26) M. Eigen and R. G. Wilkins, *Advan. Chem. Ser.*, **No. 49**, 55 (1965).

(27) K. Kustin and J. Swinehart, *Progr. Inorg. Chem.*, **13**, 107 (1970).

(28) L. J. Kirschenbaum and K. Kustin, *J. Chem. Soc. A*, 684 (1970).

(29) R. F. Pasternack, P. R. Huber, U. M. Huber, and H. Sigel, *Inorg. Chem.*, **11**, 276 (1972).

(30) K. Kustin, R. F. Pasternack, and E. M. Weinstock, *J. Amer. Chem. Soc.*, **88**, 4610 (1966).

(31) A. Kowalak, K. Kustin, R. F. Pasternack, and S. Petrucci, *ibid.*, **89**, 3126 (1967).

(32) R. F. Pasternack, M. Angwin, L. Gipp, and R. Reingold, *J. Inorg. Nucl. Chem.*, in press.

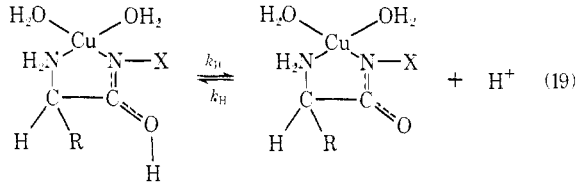
(33) However, recent work on metal dipeptides both in the solid state and in solution have been interpreted as involving copper(II)–carboxyl interactions.[18,34] This suggestion is being tested in another series of studies in our laboratory and will be reported soon.

(34) O. Yamauchi, Y. Hirano, Y. Nakao, and A. Nakahara, *Can. J. Chem.*, **47**, 3441 (1969).

for higher order complexation reactions often show some ligand dependence. Whereas the rate-determining step for this reaction is also the loss of a water molecule from the metal ion,[26] the presence of a ligand other than water in the inner coordination sphere affects the lability of the remaining water molecules.[35,36] For nickel(II) dipeptides there is little variation in lability as a comparison of the k_2/k_1 ratios demonstrates. This ratio is 2.5–4.0 for the dipeptides studied other than leucylglycine for which it is only 1.2. However, for NiL^+ of leucylglycine, at least in the solid state, there appears to be a greater than normal distortion from octahedral geometry.[18] This may be responsible in part for the somewhat unusual result for this ligand. For cobalt(II), the k_2/k_1 ratio is 0.8–1.6 for the dipeptides which have been considered to date.

The lower stability of $Cu(leugly)^+$ relative to $Cu(glygly)^+$ and $Cu(glyleu)^+$ shows up in both k_1 and k_{-1}. The k_1 for leucylglycine is a factor of 3 to 4 smaller than for the glycyldipeptides. We have already discussed some evidence that complexation for copper(II) proceeds *via* a somewhat different route that for nickel(II) or cobalt(II), *i.e.*, that copper complexation reactions may be more closely represented by an SN2 mechanism.[29] It might be anticipated, therefore, that for this metal ion, there would be more dependence of the forward rate constant on ligand characteristics than for the other metals.

The copper(II)–oligopeptide systems have attracted considerable attention because, in part, of the acidity of the complexes (*cf.* eq 15). In the case of glycylglycine[10] we found that the value of k_H is several orders of magnitude smaller than the diffusion-controlled limit. This was interpreted by us as evidence for a bonding model in which the metal ion is bonded to the terminal NH_2 group and the amide oxygen in the pH region in which the amide nitrogen is protonated. In another study, already mentioned,[18] it is suggested that the acidic proton may not be localized on the amide nitrogen but rather on the amide oxygen. The acid equilibrium could then be depicted as given in eq 19. However,



(19)

the rate constants k_H (*cf.* Table VIII) are almost independent of the particular copper–oligopeptide complex and are about four orders of magnitude smaller than the diffusion-controlled limit. It is difficult to rationalize this kinetic result with the model shown in eq 19. The protonation of the carboxyl oxygen requires no change in hybridization nor structural rearrangement. It is difficult to see why this reaction should be so slow relative to other protonations. Therefore, on the basis of kinetic evidence as well, we conclude that eq 19 is *not* correct and that the amide nitrogen is bonded to the copper ion in the Cu(L–1H) species but not in the CuL^+ species.

The rate constants k_D correlate with the acidity of the complex acid. As suggested by Hammond,[37] the correlation of rate constants with equilibrium constants for an endothermic process, such as these deprotonations,[38] implies that the transition state complex more closely resembles the products, Cu(L–1H) + H^+, than the reactants, CuL^+. However, as shown by Pagenkopf and Margerum[39] for the general acid catalysis of the formation of Cu(HGGG) from $Cu(GGG)^-$, where H_3GGG is the zwitterionic form of triglycine, the proton exchange occurs *prior* to the rate-determining step. Therefore, if these results can be applied to the dipeptide case, for the deprotonation of CuL^+, the proton is still attached to the ligand in a transition state which resembles the products more than the reactants. Thus it seems likely that a loose bond has been formed between copper and N–H in the transition state with concomitant weakening of the N–H bond and that the binding of the copper leads to the dissociation of the proton, $Cu\cdots N\cdots H \rightarrow Cu\text{–}N + H^+$. The kinetic results imply that the stable form of CuL^+ does not involve a copper to nitrogen bond but for a chelate structure a bond to the amide oxygen; this is in agreement with thermodynamic results.[9]

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(35) J. P. Hunt, *Coord. Chem. Rev.*, 7, 1 (1971).
(36) D. W. Margerum and H. M. Rosen, *J. Amer. Chem. Soc.*, 89, 1088 (1967).
(37) G. S. Hammond, *ibid.*, 77, 334 (1955).
(38) A value of $\Delta H = +7$ kcal/mol was obtained for the deprotonation reaction of $Cu(glygly)^+$: A. P. Brunetti, M. C. Lim, and G. H. Nancollas, *J. Amer. Chem. Soc.*, 90, 5120 (1968).
(39) G. K. Pagenkopf and D. W. Margerum, *ibid.*, 90, 6963 (1968).