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Ternary Complexes in Solution. XXIII. Influence of Alkyl Side Chains on the Stability of Binary and Ternary Copper(II)–Dipeptide Complexes[1]

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Equilibrium constants have been measured potentiometrically for protonation and Cu^{2+} coordination of the following dipeptides: glycylglycine, glycyl-L-alanine, L-alanylglycine, L-alanyl-L-alanine, glycyl-L-leucine, L-leucylglycine, glycyl-L-isoleucine, L-isoleucylglycine, glycylsarcosine, sarcosylglycine, glycyl-L-proline, and L-prolylglycine. Besides the binary complexes CuL^+ and Cu(L-H) the mixed-ligand complexes with 2,2'-bipyridyl, namely, Cu(bipy)L^+ and Cu(bipy)(L-H), were also studied. For the glycyl(*N*- or α-alkyl)glycinates the stability of CuL^+ depends only on the basicity of the amino group, while for the complexes with (*N*- or α-alkylglycyl)glycinates a considerable decrease in stability is observed. In contrast, with (*N*- or α-alkylglycyl)glycinates the ionization of the amide proton is facilitated; i.e., Cu(L-H) is more stable for these dipeptides than it is with glycylglycinate, while those of the glycyl-α-alkylglycinates are less stable. Roughly speaking, the results obtained for the ternary systems parallel those of the binary ones. However, in considering the former ones one must be aware of the principal influence of a second ligand: for example, the amide group in the binary Cu^{2+} complexes of glycylamide and glycylglycinate is deprotonated at pH 7 and 4, respectively, while in both corresponding and 2,2'-bipyridyl-containing ternary complexes ionization occurs at practically the same pH, i.e., about 7.7. Obviously, the bidentate 2,2'-bipyridyl prevents the coordination of the carboxylate group to the square plane of Cu^{2+} and diminishes in this way the structural differences between glycylglycine and glycylamide.

As known from crystallographic studies,[2] the initial complex formation between Cu^{2+} and peptides starts with the terminal amino group and not from the carboxylate end; i.e., a chelate is formed involving the mentioned amino moiety and the oxygen of the neighboring amide group. The same is true in aqueous solution[3,4] as can be judged from the thermodynamic[5–9] and kinetic[10,11] behavior of these complexes, which transform, roughly speaking in the neutral pH range, into complexes which are deprotonated at the amide group. In these latter species the chelate is now formed by the coordination of Cu^{2+} to the deprotonated nitrogen of the amide group.[2–11]

Thus, in peptides, and certainly also in proteins, the amide group is one of the important binding sites for the coordination of Cu^{2+}.[3] Hence, it is not surprising that the coordination behavior of peptides was also investigated in ternary complexes[6,12–14] because such mixed-ligand species may be considered as models for enzyme–metal ion–substrate complexes.[15–17] For a first approximation one may say that the behavior of peptides in the formation of ternary complexes is similar to their qualities in binary complexes. The most significant difference is, for example, in 2,2'-bipyridyl–copper(II)–oligoglycine systems, that the amide group is deprotonated rather independently on the kind of peptide while in the binary oligoglycine systems the ionization occurs in a wide pH range.[6] This is obviously the result of the coordination of the bidentate 2,2'-bipyridyl to the metal ion forcing a uniform complexation of the peptides, independent of the number of potential binding sites present.

As indicated, the initial complex formation starts in both the binary and ternary systems from the amino terminal end. Therefore, it became our aim to learn how the coordination behavior may be altered by the kind of the two terminal amino acids in such peptides or proteins. The simplest models for such studies are dipeptides; they contain all binding sites of interest. The first aim was to evaluate the influence of bulky alkyl groups on the stability and acidity of complexes formed in binary copper(II)–dipeptide and ternary 2,2'-bipyridyl–copper(II)–dipeptide systems. It turned out that depending on the position of the alkyl group in the dipeptide either the stability or the acidity of the complexes is altered. The dipeptides studied are assembled in Figure 1.

Experimental Section

Materials and Measurements. Glycylglycine, glycyl-L-alanine, L-alanylglycine, L-alanyl-L-alanine, glycyl-L-leucine, L-leucylglycine, glycyl-L-isoleucine, glycylsarcosine, glycyl-L-proline, 2,2'-bipyridyl, $NaClO_4$, and $Cu(ClO_4)_2 \cdot 6H_2O$ were purchased from Fluka AG, Buchs, Switzerland. Sarcosylglycine and L-isoleucylglycine were from Cyclo Chemical, Division Travenol Laboratories Inc., Los Angeles, Calif., and L-prolylglycine from Sigma Chemical Co., St. Louis, Mo.

The measurements were performed under N_2 by potentiometric titrations (I = 0.1, $NaClO_4$; 25°) as previously described.[6,18]

Acidity Constants of the Ligands. The acidity constants of 2,2'-bipyridyl were taken from a report by Linnell and Kaczmarczyk[19] for $K^{H}_{H_2L}$ and from the work of Anderegg[20] for K^{H}_{HL}.

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 $NaClO_4$ (I = 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* $HClO_4$ with and without ligand (8×10^{-4}, 1.2×10^{-3}, or 1.6×10^{-3} *M*) using 1 ml of 0.1 *M* NaOH (I = 0.1, $NaClO_4$; 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 at least 4 and at least 25 independent titrations, respectively.

Stability and Acidity Constants of the Binary and Ternary Complexes. The conditions 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; [dipeptide] = 8×10^{-4} *M*), but a part of $NaClO_4$ was replaced by $Cu(ClO_4)_2$ with the ratio Cu:L = 1:1. Usually six titrations were carried out for each system. As in several cases in the 1:1 system the species CuL^+ occurred only in insignificant amounts; for all systems (including glycylglycine which was already studied earlier[6]) 10 additional titrations were made with a Cu:L ratio between 5:1 and 40:1. Titrations of solutions without ligand (and Cu^{2+}) were used as a basis for the evaluation.[6,21]

Similarly, the constants $K^{Cu(bipy)}_{Cu(bipy)L}$ and $K^{H}_{Cu(bipy)L}$ were determined from usually seven titrations with Cu:bipy:L = 1:1:1 and from at least six titrations with Cu:bipy:L = 2:2:1 or 4:4:1.[6,21] The stability constants of the copper(II)–2,2'-bipyridyl 1:1 and 1:2 complexes, which were used for the evaluation of the mixed-ligand systems, were taken from the work of Anderegg.[20]

The stability constants due to the binary and ternary systems containing glycylsarcosine and glycyl-L-proline, where no amide proton is liberated, were determined as described earlier.[18]

Results

The constants due to equilibria 3 and 4 which correspond to the binary copper(II)–dipeptide systems were computed from potentiometric titrations (I = 0.1, $NaClO_4$; 25°) taking into account the species H^+, H_2L^+, HL, L^-, Cu^{2+}, CuL^+, and

Table I. Acidity Constants of Some Dipeptides and of Their Corresponding Binary Cu^{2+} Systems ($I = 0.1$, $NaClO_4$; 25°)[a]

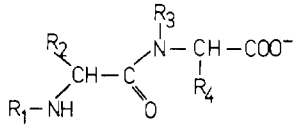
Dipeptide	$pK^{H}_{H_2L}$	pK^{H}_{HL}	$pK^{2H}_{Cu+H_2L}$	$pK^{3H}_{Cu+H_2L}$
Glycinamide[22]		8.04		
Glycylglycine	3.11 ± 0.01	8.15 ± 0.01	5.71 ± 0.07	9.70 ± 0.03
Glycyl-L-alanine	3.17 ± 0.02	8.25 ± 0.01	5.63 ± 0.07	9.67 ± 0.04
L-Alanylglycine	3.20 ± 0.01	8.17 ± 0.01	6.11 ± 0.07	9.75 ± 0.03
Glycyl-L-leucine[11]	3.13 ± 0.01	8.28 ± 0.01	5.52 ± 0.05	10.28 ± 0.06
L-Leucylglycine[11]	3.05 ± 0.01	8.10 ± 0.01	6.40 ± 0.22	9.66 ± 0.05
Glycyl-L-isoleucine	3.11 ± 0.01	8.26 ± 0.01	5.54 ± 0.08	10.25 ± 0.08
L-Isoleucylglycine	3.15 ± 0.01	8.07 ± 0.01	6.47 ± 0.16	9.73 ± 0.03
Glycylsarcosine	2.78 ± 0.01	8.59 ± 0.01	5.03[b]	
Sarcosylglycine	3.08 ± 0.01	8.54 ± 0.01	6.30 ± 0.09	10.26 ± 0.04
Glycyl-L-proline	2.79 ± 0.02	8.55 ± 0.01	4.84[b]	
L-Prolylglycine	3.15 ± 0.01	8.98 ± 0.01	5.71 ± 0.09	9.47 ± 0.06
L-Alanyl-L-alanine	3.25 ± 0.01	8.19 ± 0.01	6.06 ± 0.10	9.57 ± 0.04

[a] The range of error given is 3 times the standard deviation. [b] Calculated from the other data given in Tables I and II.

Table II. Equilibrium Constants of Some Binary Copper(II)–Dipeptide and Ternary 2,2'-Bipyridyl–Copper(II)–Dipeptide Systems ($I = 0.1$, $NaClO_4$; 25°)[a]

Dipeptide	$\log K^{Cu}_{CuL}$	pK^{H}_{CuL}	$\log^{Cu(bipy)}_{Cu(bipy)L}$	$pK^{H}_{Cu(bipy)L}$	$\Delta \log K$
Glycinamide[22]	5.40	7.01	5.01	7.71	−0.39
Glycylglycine	5.55 ± 0.07	3.99 ± 0.06	5.09 ± 0.10	7.77 ± 0.04	−0.46
Glycyl-L-alanine	5.79 ± 0.07	4.04 ± 0.08	5.61 ± 0.05	7.84 ± 0.15	−0.18
L-Alanylglycine	5.26 ± 0.07	3.64 ± 0.06	4.51 ± 0.26	6.29 ± 0.02	−0.8
Glycyl-L-leucine	5.89 ± 0.05[11]	4.76 ± 0.06[11]	5.75 ± 0.03	8.58 ± 0.03	−0.14
L-Leucylglycine	4.75 ± 0.22[11]	3.26 ± 0.23[11]	4.13 ± 0.10	6.33 ± 0.04	−0.6
Glycyl-L-isoleucine	5.83 ± 0.08	4.71 ± 0.03	5.79 ± 0.03	8.66 ± 0.02	−0.04
L-Isoleucylglycine	4.75 ± 0.16	3.26 ± 0.17	4.18 ± 0.10	6.29 ± 0.02	−0.6
Glycylsarcosine	6.34 ± 0.01[b]		6.21[c,f]		−0.13
Sarcosylglycine	5.32 ± 0.09	3.96 ± 0.11	4.32 ± 0.08	6.60 ± 0.05	−1.00
Glycyl-L-proline	6.50 ± 0.01[d]		6.43[e,f]		−0.07
L-Prolylglycine	6.42 ± 0.09	3.76 ± 0.13	5.39 ± 0.19	6.44 ± 0.03	−1.0
L-Alanyl-L-alanine	5.38 ± 0.10	3.51 ± 0.09	5.06 ± 0.12	7.08 ± 0.02	−0.3

[a] The range of error given is 3 times the standard deviation. [b] $\log K^{CuL}_{CuL_2} = 5.14 \pm 0.03$. [c] Calculated from $\log \beta^{Cu}_{Cu(bipy)L} = 14.21 \pm 0.01$. [d] $\log K^{CuL}_{CuL_2} = 5.13 \pm 0.02$. [e] Calculated from $\log \beta^{Cu}_{Cu(bipy)L} = 14.43 \pm 0.01$. [f] $\log K^{Cu(bipy)}_{Cu(bipy)L} = \log \beta^{Cu}_{Cu(bipy)L} - \log K^{Cu}_{Cu(bipy)}$.



$R_1 = R_2 = R_3 = R_4 = H$: glycylglycinate (gg)

$R_1 = R_2 = R_3 = H$
$R_4 = CH_3$: glycyl-L-alaninate (ga)
$= CH_2CH(CH_3)_2$: glycyl-L-leucinate (gl)
$= CH(CH_3)CH_2CH_3$: glycyl-L-isoleucinate (gi)

$R_1 = R_3 = R_4 = H$
$R_2 = CH_3$: L-alanylglycinate (ag)
$= CH_2CH(CH_3)_2$: L-leucylglycinate (lg)
$= CH(CH_3)CH_2CH_3$: L-isoleucylglycinate (ig)

$R_1 = R_3 = H$; $R_2 = R_4 = CH_3$: L-alanyl-L-alaninate (aa)

$R_1 = R_2 = R_4 = H$; $R_3 = CH_3$: glycylsarcosinate (gs)

$R_2 = R_3 = R_4 = H$; $R_1 = CH_3$: sarcosylglycinate (sg)

$R_1 = R_2 = H$; R_3–$R_4 = CH_2CH_2CH_2$: glycyl-L-prolinate (gp)

$R_3 = R_4 = H$; R_1–$R_2 = CH_2CH_2CH_2$: L-prolylglycinate (pg)

Figure 1. List of dipeptides used in this study.

Cu(L-H).

$$H_2L^+ \rightleftharpoons HL + H^+ \quad (1)$$

$$K^{H}_{H_2L} = [HL][H]/[H_2L]$$

$$HL \rightleftharpoons L^- + H^+ \quad (2)$$

$$K^{H}_{HL} = [L][H]/[HL]$$

$$Cu^{2+} + H_2L^+ \rightleftharpoons CuL^+ + 2H^+ \quad (3)$$

$$K^{2H}_{Cu+H_2L} = [CuL][H]^2/[Cu][H_2L]$$

$$Cu^{2+} + H_2L^+ \rightleftharpoons Cu(L\text{-}H) + 3H^+ \quad (4)$$

$$K^{3H}_{Cu+H_2L} = [Cu(L\text{-}H)][H]^3/[Cu][H_2L]$$

The connection between eq 3 and 4 and the commonly used constants as defined by eq 5 and 6 is given by eq 7 and 8.

$$Cu^{2+} + L^- \rightleftharpoons CuL^+ \quad (5)$$

$$K^{Cu}_{CuL} = [CuL]/[Cu][L]$$

$$CuL^+ \rightleftharpoons Cu(L\text{-}H) + H^+ \quad (6)$$

$$K^{H}_{CuL} = [H][Cu(L\text{-}H)]/[CuL]$$

$$\log K^{Cu}_{CuL} = pK^{H}_{H_2L} + pK^{H}_{HL} - pK^{2H}_{Cu+H_2L} \quad (7)$$

$$pK^{H}_{CuL} = pK^{3H}_{Cu+H_2L} - pK^{2H}_{Cu+H_2L} \quad (8)$$

The evaluation of the ternary 2,2'-bipyridyl–copper(II)–dipeptide systems was done taking into account the species already mentioned *plus* $H_2(bipy)^{2+}$, $H(bipy)^+$, bipy, $Cu(bipy)^{2+}$, $Cu(bipy)_2^{2+}$, and the two mixed-ligand complexes $Cu(bipy)L^+$ and Cu(bipy)(L-H). In this case, the constants due to eq 9 and 10 were computed directly.

$$Cu(bipy)^{2+} + L^- \rightleftharpoons Cu(bipy)L^+ \quad (9)$$

$$K^{Cu(bipy)}_{Cu(bipy)L} = [Cu(bipy)L]/[Cu(bipy)][L]$$

$$Cu(bipy)L^+ \rightleftharpoons Cu(bipy)(L\text{-}H) + H^+$$

$$K^{H}_{Cu(bipy)L} = [H][Cu(bipy)(L\text{-}H)]/[Cu(bipy)L] \quad (10)$$

The acidity constants of the dipeptides (including glycinamide)[22] and of their binary Cu^{2+} systems (eq 1–4) are summarized in Table I. Table II contains the equilibrium constants determined for the binary copper(II)–dipeptide (eq 5–8) and ternary 2,2'-bipyridyl–copper(II)–dipeptide complexes (eq 9 and 10), together with the values for $\Delta \log K$. This latter constant is defined by eq 11 and corresponds to equilibrium 12; it characterizes the stability of ternary complexes.[15–18]

$$\Delta \log K = \log K^{Cu(bipy)}_{Cu(bipy)L} - \log K^{Cu}_{CuL} = \log K^{CuL}_{CuL(bipy)} - \log K^{Cu}_{Cu(bipy)} \quad (11)$$

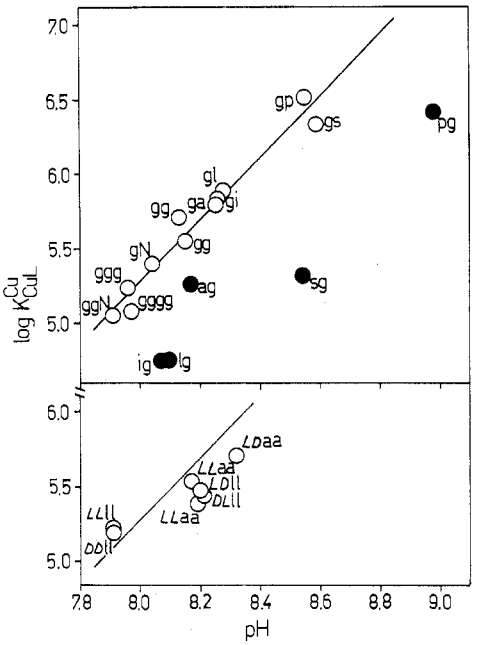


Figure 2. Relation between log K^{Cu}_{CuL} and pK^{H}_{HL} for the *binary* 1:1 complexes CuL^+. Upper part: the oligoglycines (gN, glycinamide; gg, glycylglycinate, etc.; see ref 6), the glycyl(*N*- or α-alkyl)glycinates (○), and the (*N*- or α-alkylglycyl)glycinates (●) (for abbreviations and data see Figure 1 and Tables I and II,, respectively). Lower part: the optical isomers of alanylalaninate and leucylleucinate (cf. Table III).[7] The reference line is taken from the upper part of the figure.

$$CuL^+ + Cu(bipy)^{2+} \rightleftharpoons Cu(bipy)L^+ + Cu^{2+} \quad (12)$$

The constants due to equilibria 3 and 4 (Table I) for the copper(II)–glycyl-α-alkylglycinate systems could easily be determined. However, in the copper(II)–(*N*- or α-alkylglycyl)glycinate systems [CuL^+] is always rather low. This means the amino and the amide protons are set free in nearly one step according to equilibrium 4. As a result the concentration of Cu(L-H) is in excess of the concentration of CuL^+. Certainly, with the aid of experiments using a large excess of Cu^{2+} (cf. Experimental Section) the situation could be somewhat improved, but still the experimental error in the constant of equilibrium 3 is commonly rather large, while that in the constant of equilibrium 4 is usually much smaller. Similar arguments hold for the mixed-ligand systems, i.e., for equilibria 9 and 10 (Table II).

Some of the binary copper(II)–dipeptide systems have already been studied earlier and the data[9,23–27] agree well with the present results, with the exception of some copper(II)–(*N*- or α-alkylglycyl)glycine systems.[25–27] However, the reasons for these discrepancies have been discussed in detail recently[11] and shall not be repeated now.[28]

Discussion

Stability of the Binary Complexes CuL^+. As mentioned in the introduction the initial complex formation between Cu^{2+} and a peptide results in a chelate involving the terminal amino moiety and the oxygen of the neighboring amide group.[30] Hence, the kind of the second (bifunctional) amino acid in a peptide should have little influence on the stability of the complexes. In accord herewith Rabin[32] has observed a straight line by plotting log K^{Cu}_{CuL} vs. pK^{H}_{HL} for a number of glycylpeptides; this means the stability of the complexes depends only on the basicity of the terminal amino group.[33] This is confirmed by the present results: the data from Tables I and II are plotted for the dipeptides of Figure 1 and for several oligoglycines from earlier work[6] in the upper part of Figure 2.

Table III. Comparison of the Acidity Constants and of the Equilibrium Constants for the Binary Cu^{2+} Complexes of the Optical Isomers of Alanylalanine and Leucylleucine[7] with the Corresponding Data for Glycylglycine and for the Related Glycyl-α-alkylglycines or the (α-Alkylglycyl)glycines

Dipeptide	pK^{H}_{HL}	log K^{Cu}_{CuL}	pK^{H}_{CuL}
Glycylglycine	8.15	5.55	3.99
L-Alanylglycine	8.17	5.26	3.64
Glycyl-L-alanine	8.25	5.79	4.04
L-Alanyl-L-alanine (LLaa)	8.19	5.38	3.51
	8.17[7]	5.54[7]	3.72[7]
L-Alanyl-D-alanine (LDaa)[7]	8.32	5.71	3.96
L-Leucylglycine	8.10	4.75	3.26
Glycyl-L-leucine	8.28	5.89	4.76
L-Leucyl-L-leucine (LLll)[7]	7.91	5.21	3.88
D-Leucyl-D-leucine (DDll)[7]	7.91	5.20	3.90
L-Leucyl-D-leucine (LDll)[7]	8.20	5.48	4.88
D-Leucyl-L-leucine (DLll)[7]	8.21	5.45	4.89

Evidently the glycyl(*N*- or α-alkyl)glycines and the oligoglycines fit the straight line of Figure 2 within the experimental error quite well. However, the (*N*- or α-alkylglycyl)glycinate–copper(II) complexes are by about 0.4–1.1 log units less stable than is expected on the basis of the basicity of the terminal amino group of the corresponding dipeptides. This indicates clearly that an alkyl substituent at the glycine end of a dipeptide has no influence on the stability of the complexes CuL^+, while such a substituent at the glycyl residue leads to a distinct decrease in stability, i.e., exhibits "steric effects".[34] A comparison of the L-alanylglycinate system with the one of sarcosylglycinate shows, as one would expect, that the "steric" influence of a methyl group in the α position is less pronounced than the one of a methyl group directly substituted at the amino group.

It is interesting to compare the present results with those obtained by Nakon and Angelici[7] for the Cu^{2+} complexes of the optical isomers of alanylalanine and leucylleucine (cf. Table III). The comparison shows a similar stability of CuL^+ for glycyl-L-alanine and L-alanyl-D-alanine or for glycyl-L-leucine and L-leucyl-D-leucine. In other words the lower stability observed for the Cu^{2+} complexes with L-alanylglycine and L-leucylglycine is diminished by the presence of an additional bulky group in the D-aminoacetate moiety.

This becomes even more evident from the lower part of Figure 2 where the reference line taken from the upper part of the figure is compared with the data of the Cu^{2+} complexes obtained for the optical isomers of alanylalanine and leucylleucine. Obviously, the corresponding points are rather close to the reference line; this means the stability of these complexes appears to be governed by the basicity of the amino group,[35] while the influence of the side chains is diminished. The presence of an alkyl side chain in each amino acid residue of a dipeptide leads apparently to a configuration that is equally suitable for complexation than is the configuration of glycylglycine. Again, with a *single* side chain this was found only in case of the glycyl(*N*- or α-alkyl)glycinates and *not* with the (*N*- or α-alkylglycyl)glycinates, where "steric effects" are observed (cf. upper part of Figure 2).

Stability of the Ternary Complexes $Cu(bipy)L^+$. To a first approximation one may conclude that the influence of alkyl substituents on the stability of the ternary complexes $Cu(bipy)L^+$ parallels the observations made with the binary species CuL^+ (cf. Tables I and II). A more detailed consideration may be made from Figure 3 where log $K^{Cu(bipy)}_{Cu(bipy)L}$ is plotted vs. pK^{H}_{HL} for the dipeptides of Figure 1 and several 2,2'-bipyridyl–copper(II)–oligoglycine systems, studied earlier.[6] There appear to be two possible interpretations: (i) the glycyl(*N*- or α-alkyl)glycinate systems fit on a straight line together with the oligoglycines (dashed line in Figure 3). (ii) the oligoglycine systems fit a straight

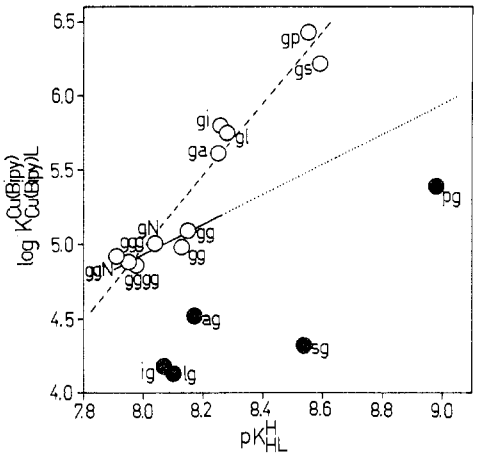


Figure 3. Relation between log $K^{Cu(bipy)}{}_{Cu(bipy)L}$ and $pK^{H}{}_{HL}$ for the *ternary* 1:1:1 complexes $Cu(bipy)L^+$ where L = oligoglycine (gN, glycinamide; gg, glycylglycinate, etc.; see ref 6), glycyl(*N*- or α-alkyl)glycinate (○), or (*N*- or α-alkylglycyl)glycinate (●) (for abbreviations and data see Figure 1 and Tables I and II, respectively).

line by themselves and then the scattering of the data is considerably less (full and dotted lines in Figure 3). Both interpretations have in common that they demonstrate a lower stability of the (*N*- or α-alkylglycyl)glycinate systems, but (ii) indicates in addition an increased stability for the glycyl(*N*- or α-alkyl)glycinate dipeptide complexes.

Even though there remains some doubt, as the reference line due to the oligoglycines is so short, we favor this second interpretation for the following reason: the values of $\Delta \log K$ (eq 11) which characterize equilibrium 12 are commonly in the order of about −0.4 log unit[36] as is also observed for the unsubstituted glycinamide and glycylglycinate systems (cf. Table II), while for the glycyl(*N*- or α-alkyl)glycinate systems $\Delta \log K \simeq -0.1$. This means these mixed-ligand complexes are indeed somewhat more stable than is usually observed for Cu^{2+} complexes formed by 2,2'-bipyridyl and a ligand with an O and N donor.[36] The lower stability of the ternary complexes formed with (*N*- or α-alkylglycyl)glycinates is also evident from $\Delta \log K \simeq -0.8$.

A comparison of the stability of $Cu(bipy)L^+$ formed with glycyl-L-alanine, L-alanylglycine, L-alanyl-L-alanine, and glycylglycine (Table II) evidences again the mutual diminishing influence of two alkyl groups. The stability of these complexes decreases within the series where L = glycyl-L-alanine > glycylglycine ~ L-alanyl-L-alanine > L-alanylglycine.

Stability of the Amide-Deprotonated Binary Species Cu(L-H). At higher pH, the complex CuL^+, where the dipeptide is bound in a bidentate manner, undergoes deprotonation of the amide group and rearrangement to the tridentate chelate Cu(L-H). Hence, in this latter species the dipeptide is coordinated via the amino residue, the nitrogen of the amide moiety, and the carboxylate group.[2,6,7] This is confirmed by a comparison of the glycinamide and glycylglycinate systems with $pK^{H}{}_{CuL} = 7.0$ and 4.0, respectively (cf. Table II): the presence of the carboxylate group allows the formation of a tridentate chelate after ionization of the amide proton and favors therefore this ionization by a factor of 10^3.

It is now interesting to compare the glycylglycine system with the other dipeptides of Table II. Obviously, substitution of an alkyl group in the glycyl moiety favors ionization of the amide proton, while substitution in the glycinate residue lowers the deprotonation tendency.[37] In other words the acidity of the complex CuL^+ decreases within the series where L = (*N*- or α-alkylglycyl)glycinate > glycylglycinate > glycyl-α-alkylglycinate.[38]

In case of the leucyl-containing dipeptides, where large bulky group differences are more significant, it is worthwhile to include into the consideration also the optical isomers of leucylleucine studied by Nakon and Angelici.[7] Again it turns out that the values of $pK^{H}{}_{CuL}$ (cf. Table III) are very similar for the glycyl-L-leucine and L-leucyl-D-leucine (or D-leucyl-L-leucine) systems. Additionally, CuL^+ of the "pure" isomers is more acidic than that of the preceding "mixed" isomers but quite similar to the one formed with glycylglycine, while the system with L-leucylglycine is still more acidic. Hence again, the presence of a second bulky group diminishes the influence of the first one. These observations reflect probably different preferred configurations,[39] which depend upon location, size, and shape of the side-chain group in these peptide systems.[7,14]

Stability of the Amide-Deprotonated Ternary Species Cu(bipy)(L-H). In discussing the stability of the mixed-ligand species Cu(bipy)(L-H) one must first note that the values of $pK^{H}{}_{Cu(bipy)L}$ for glycinamide and glycylglycinate are identical within experimental error (=7.7; cf. Table II). This means, contrary to the observations in the binary system, that the carboxylate group is now without an influence on the stability of Cu(bipy)(L-H). However, this is easily rationalized by taking into account that two of the four positions of the square plane around Cu^{2+} are occupied by 2,2'-bipyridyl in the ternary complex; as a result the carboxylate group cannot participate here anymore.

By using the glycylglycine system with $pK^{H}{}_{Cu(bipy)L} = 7.77$ for further comparisons one arrives at the conclusions already given for the binary systems: an alkyl substituent at the glycyl residue of a dipeptide facilitates the ionization of the amide proton, while the same substituent at the glycinate group lowers the tendency for ionization.

The stability of the complexes Cu(bipy)(L-H) and $Cu(bipy)L^+$ where L = L-alanyl-L-alanine is between those of the corresponding complexes formed with L-alanylglycine or glycyl-L-alanine.

General Considerations. Even though the interpretation of some of the results appears at present not unambiguously possible, especially the interpretation of the observation that the substitution of a second alkyl group in a monosubstituted dipeptide diminishes to a large part the influence of the first one, the distinct influence of bulky groups on the stability of peptide complexes is definite.[40] Similarly, the reactivity is altered also as shown in a study by Margerum et al.[14] on the kinetics of copper(II)–tripeptide reactions. For example, the rate constant for the nucleophilic substitution reaction of triethylenetetramine with $Cu(L\text{-}2H)^-$, where L = glycyl-L-leucylglycinate is a factor of >200 smaller than the rate constant for the same reaction where L = glycylglycylglycinate. Substitution of leucinate for glycinate in the carboxylate terminal residue is only slightly less effective in slowing the rate of the nucleophilic trien attack, while substitution for the amine terminal residue of the tripeptide is relatively ineffective.

Finally, it is worthwhile to correlate in some examples the stability of complexes with their concentration in solution. Let us first consider the more simple case where no amide proton ionization can occur and compare the formation of a ternary complex with the corresponding binary one. For this reason, the distributions of the several complex species as dependent upon pH were calculated for the binary copper(II)–glycylsarcosine 1:1 system and for the ternary 2,2'-bipyridyl–copper(II)–glycylsarcosine 1:1:1 system (cf. Figure 4). The concentrations of all species tend to approach certain constant values with increasing pH. The concentration of the ternary complex, Cu(bipy)(glycylsarcosinate)$^+$, goes up to 93% of the total Cu^{2+} present in solution, while in the binary system the complex Cu(glycylsarcosinate)$^+$ reaches only about 66%. Thus, in the binary system, the concentrations of Cu^{2+} and the 1:2 complex are still reasonably high (each about 17%), while in

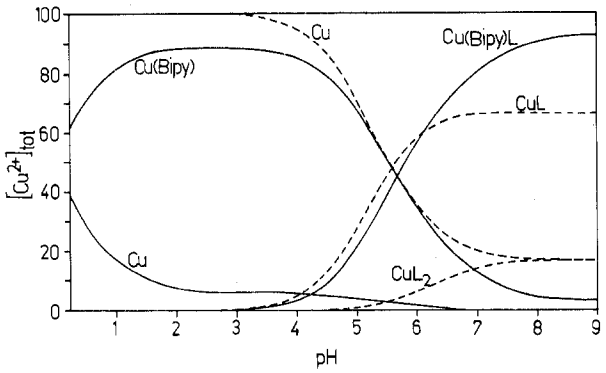


Figure 4. Comparison of the influence of pH on the concentrations (given as the percentage of the total Cu^{2+} present) of the several species present in an aqueous solution of the *binary* system (broken lines) Cu^{2+} and glycylsarcosine (each 10^{-3} *M*) and of the *ternary* system (solid lines) Cu^{2+}, 2,2'-bipyridyl, and glycylsarcosine (each 10^{-3} *M*)—computed with the constants given in Tables I and II. The sum of the concentrations of $Cu(bipy)_2^{2+}$, CuL^+, and CuL_2 in the ternary system is always less than 7.5%. Hydrolysis was omitted in these calculations.

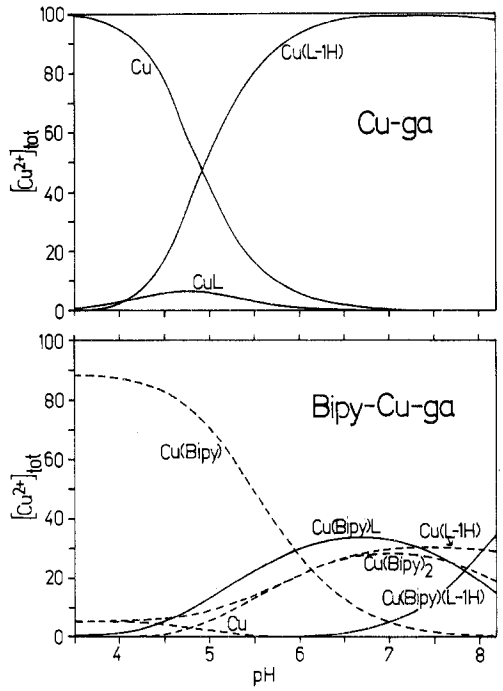


Figure 5. Influence of pH on the concentrations (given as the percentage of the total Cu^{2+} present) of the several species present in an aqueous solution of the *binary* system (*upper* part) Cu^{2+} and glycyl-L-alanine (each 10^{-3} *M*) and of the *ternary* system (*lower* part) Cu^{2+}, 2,2'-bipyridyl, and glycyl-L-alanine (each 10^{-3} *M*)—computed with the equilibrium constants of Tables I and II. The concentration of CuL^+ in the ternary system is <1%; the concentration of hydrolyzed species is <3% in the binary and ternary system at pH ⩽8.

the mixed-ligand system the ternary complex dominates very strongly.

Another example which demonstrates the influence of a ligand like 2,2'-bipyridyl on the distribution of complexes formed in a copper(II)–dipeptide system is shown in Figure 5. Here glycyl-L-alanine was used where an ionizable amide proton is present. Again, the influence of 2,2'-bipyridyl is dramatic but affects the system now in the opposite sense as in the preceding example. While in the binary system in certain pH regions the concentration of one complex strongly dominates, this is no longer true in the presence of 2,2'-bipyridyl where at all pH values a number of species are in equilibrium.

As discussed, in both the binary CuL^+ and the ternary

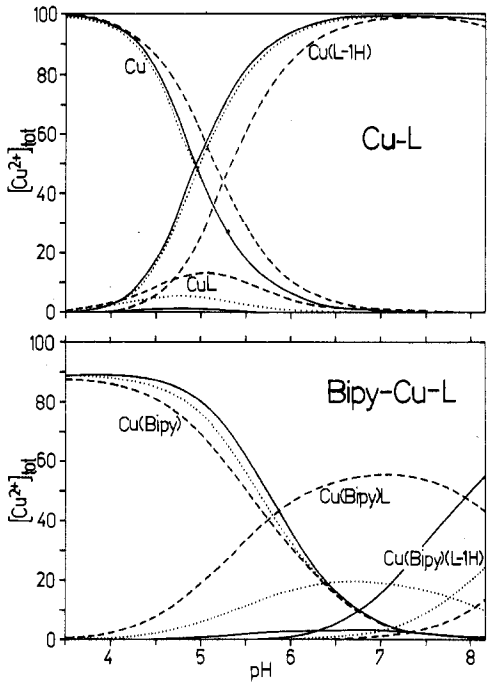


Figure 6. Comparison of the influence of pH on the concentrations (given as the percentage of the total Cu^{2+} present) of the *binary* complexes CuL^+ and Cu(L-H) (*upper* part) and of the mixed-ligand complexes $Cu(bipy)L^+$ and Cu(bipy)(L-H) (*lower* part), present in an aqueous solution where L = L-isoleucylglycinate (solid lines), glycyl-L-isoleucinate (broken lines), or glycylglycinate (dotted lines). The computation made use of the constants of Tables I and II and concentrations of 10^{-3} *M* for each reactant.

$Cu(bipy)L^+$ complexes a bulky group at or neighboring the amino group lowers the stability. On the contrary, it also favors the loss of a proton from the amide binding site and, thus, enhances the stability of the complexes Cu(L-H) and Cu(bipy)(L-H). This is evidently reflected in the distribution curves shown in Figure 6 for the binary and ternary systems containing glycylglycine, glycyl-L-isoleucine, or L-isoleucylglycine. In addition it should be noted that in the mixed-ligand system the discrimination behavior originated by the bulky group is much more pronounced than in the binary system and hence the species $Cu(bipy)L^+$ may occur in significantly larger concentrations than the corresponding species CuL^+ in the binary system. Thus one may conclude that "steric alterations" are possibly important in metal ion–protein interactions as the concentration of a species may in this way not only be lowered but also be increased.

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Registry No. Glycylglycine, 556-50-3; glycyl-L-alanine, 3695-73-6; L-alanylglycine, 687-69-4; glycyl-L-isoleucine, 19461-38-2; L-isoleucylglycine, 868-28-0; glycylsarcosine, 29816-01-1; sarcosylglycine, 38082-72-3; glycyl-L-proline, 704-15-4; L-prolylglycine, 2578-57-6; L-alanyl-L-alanine, 1948-31-8; $Cu(gg)^+$, 54964-29-3; $Cu(ga)^+$, 54964-31-7; $Cu(ag)^+$, 54964-30-6; $Cu(gi)^+$, 54964-32-8; $Cu(ig)^+$, 54964-33-9; $Cu(gs)^+$, 54964-34-0; $Cu(sg)^+$, 54964-18-0; $Cu(gp)^+$, 54964-35-1; $Cu(pg)^+$, 54964-17-9; $Cu(aa)^+$, 54964-36-2; $Cu(bipy)(gg)^+$, 54964-22-6; $Cu(bipy)(ga)^+$, 54964-21-5; $Cu(bipy)(ag)^+$, 54964-23-7; $Cu(bipy)(gl)^+$, 54964-24-8; $Cu(bipy)(lg)^+$, 54964-25-9;

Cu(bipy)(gi)$^+$, 54964-20-4; Cu(bipy)(ig)$^+$, 54964-26-0; Cu(bipy)(gs)$^+$, 54964-19-1; Cu(bipy)(sg)$^+$, 54964-15-7; Cu(bipy)(gp)$^+$, 54964-27-1; Cu(bipy)(pg)$^+$, 54964-16-8; Cu(bipy)(aa)$^+$, 54964-28-2.

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(33) The effect of the alkyl groups on the acid ionization constants is relatively small; this is in accord with the observation at several dipeptides made by Nancollas et al.[9] These authors have determined values for ΔH and ΔS, and they conclude: "The driving force in the protonation of the carbonyl group is entirely entropic in origin. Conversely, the enthalpy of protonation of the amino group is large and negative while the entropy term is small."
(34) This term is used here in a rather general sense; it is not intended to restrict its meaning solely on changes in bond strength but to include also such factors as solvation changes[8,9] or reduced formation rates (k_1) due to screening by the alkyl groups. (E.g.: the lower stability of Cu(lg)$^+$ relative to Cu(gg)$^+$ and Cu(gl)$^+$ shows up in k_1 and k_{-1}. The k_1 for leucylglycinate is a factor 3–4 smaller than for gg or gl.)[11] Obviously, for a separation of effects the enthalpy and entropy terms must be known in addition to ΔG, as well as the detailed kinetics of the complexation reactions.
(35) The influence of the conformation on the acidity constants of "pure" L,L and "mixed" L,D dipeptides has been discussed by Nakon and Angelici.[7] In this context the cis–trans conformational dependence of the carboxylate pK (trans, 3.01; cis, 3.39) in glycylsarcosine as observed by R. A. Morton and S. S. Danyluk, *Can. J. Chem.*, **52**, 2348 (1974), should also be mentioned.
(36) Usually $\Delta \log K \simeq -0.4$ for complexes formed with Cu^{2+}, 2,2'-bipyridyl and a ligand with an O and N donor.[16-18]
(37) Recently, in private correspondence Professor Robert J. Angelici raised in this connection the following question: "Is it possible that the high values of pK^{H}_{CuL} for the glycyl-α-alkylglycinates are due to steric interaction with an axial water ligand? This chelate ring is planar and quite rigid, so the side chain cannot bend away." Indeed, preliminary results of dipeptides with an additional donor group in the side chain, like glycyl-L-methionine, indicate an increased stability of Cu(L-H), possibly due to the coordination of the third function.
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