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The Stability of Fused Rings in Metal Chelates. VIII. Solution Equilibria of the Copper(II) Complexes of Glycinamide and Related Compounds

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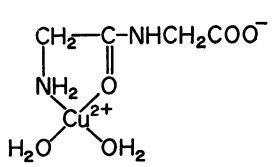
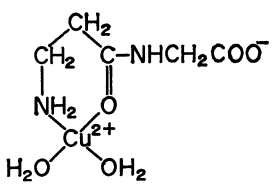
The aqueous solution equilibria of the systems containing copper(II) ion and glycinamide, β-alaninamide, glycine-*N*,*N*-diethylamide, or β-alanine-*N*,*N*-diethylamide have been investigated by potentiometric titration at 25°C (μ=0.1 (KNO_3)). The stability constants K_1 and K_2 and the constants of the deprotonation reactions K_1^H, K_{c1}, and K_{c2}, for the glycinamide-copper(II) systems, and K_1 and K_2 for the other ligand-copper(II) systems were calculated by the method of non-linear least-squares with the aid of a computer. The K_1 values were found to be in the order, glycine-*N*,*N*-diethylamide (6.17)>β-alanine-*N*,*N*-diethylamide (5.5)>glycinamide (5.30)>β-alaninamide (5.1). The sequence reveals that the five-membered chelates are more stable than the six-membered ones. The K_1 and K_{c1} values for glycylglycine and β-alanylglycine have been determined anew, and the structures of the chelates formed in acid solution have been discussed from comparative studies of the equilibrium constants, which suggest a tridentate nature of the dipeptides in the chelates of the type $Cu(HL)^+$ where HL denotes free dipeptide.

From comparative studies of the stability constants of various copper(II)-dipeptide chelates, Rabin[1] postulated that in acid solution glycylglycine (Gly·Gly) binds with copper(II) as bidentate ligand through the amino nitrogen and peptide oxygen as indicated by the structure Ia. Since then a considerable number of papers have appeared dealing with the nature of the copper(II)-peptide bonding in the chelates formed in acid solution.

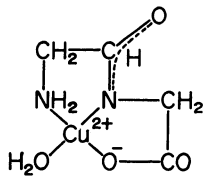


Ia

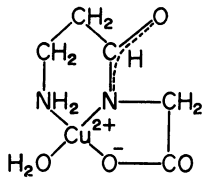


Ib

Nancollas and his co-workers[2-4] confirmed the bidentate nature of Gly·Gly, triglycine, and tetraglycine on the basis of thermodynamic investigations, and



IIa



IIb

1) B. R. Rabin, *Trans. Faraday Soc.* **52**, 1130 (1956).

2) A. P. Brunetti, M. C. Lim and G. H. Nancollas, *J. Amer. Chem. Soc.*, **90**, 5120 (1968).

3) G. H. Nancollas and D. J. Poulton, *Inorg. Chem.*, **8**, 680 (1969).

4) G. H. Nancollas, *Coord. Chem. Rev.*, **5**, 407 (1970).

Pagenkopf and Margerum[5] and Pasternack *et al.*[6] also obtained results supporting the view from kinetic studies. Kim and Martell,[7,8] on the other hand, favored the fused-ring structures like IIa from the potentiometric and spectral points of view.

We reported[9] the reactions of copper(II) with some dipeptides composed of glycine and/or β-alanine as studied from the viewpoint of the relative stability of fused-ring chelates, and interpreted the results as indicative of the coordination to copper(II) in acid solution by the amino nitrogen, amide nitrogen, and carboxyl oxygen of the dipeptides as shown by IIa and IIb.

The necessity for further information on the bonding modes in solution prompted us to carry out studies on the interactions of copper(II) with some amino acid amides as fundamental constituents of peptides. In this connection, it seemed significant to investigate comparatively the stabilities and structures of the copper(II) complexes of glycinamide, β-alaninamide, and the corresponding *N,N*-diethylamides in order to get information on the contribution of peptide oxygen and nitrogen atoms to coordination.

The solution equilbria of the copper(II)-glycinamide complexes have been reported by Datta and Rabin,[10] Li *et al.*,[11] and Sigel.[12] Regardh[13] carried out detailed studies on the equilibria involved in a 2 : 1 glycinamide-copper(II) mixture as well as the copper(II) ion-catalyzed hydrolysis of glycinamide, and obtained equilibrium constants by the method of Bjerrum. However, the equilibrium constants for glycinamide reported so far by these investigators were determined under different conditions and do not seem to be complete. It might be of value to reinvestigate the glycinamide-copper(II) systems along with the other amide-copper(II) systems.

This paper deals with the studies on the solution equilibria of the copper(II) complexes of glycinamide (GA), β-alaninamide (β-AA), glycine-*N,N*-diethylamide (GDA), and β-alanine-*N,N*-diethylamide (β-ADA), and a discussion on the structures and stabilities of the complexes.

Experimental

Reagents. GA hydrochloride was purchased from Nakarai Chemicals Co., Ltd., and used without further purification. β-AA hydrochloride,[14] GDA hydrochloride,[15] Gly·Gly,[16,17] and β-alanylglycine (β-Ala·Gly)[18] were prepared according to literature and checked by the melting point and elemental analysis.

*β-Alanine-*N,N*-diethylamide* (β-ADA). To a stirred solution of diethylamine (6.6 g, 90 mmol) in toluene (50 m*l*) was added, under cooling in ice water, carbobenzoxy-β-alanyl chloride[19] in toluene (50 m*l*) prepared from carbobenzoxy-β-alanine (10.0 g, 45 mmol), and the mixture was stirred for 2.5 hr at room temperature. The filtrate from diethylamine hydrochloride was washed successively with dilute aqueous sodium carbonate and water and dried over magnesium sulfate. The yellow oil obtained after evaporation of the solvent *in vacuo* was reduced with Pd-H_2 in methanol (250 m*l*) containing concentrated hydrochloric acid (2 m*l*). After removal of the catalyst and solvent, the oily residue was dried over P_2O_5 *in vacuo* and crystallized by washing with acetone. Yield, 3.0 g. Recrystallization from ethanol-acetone-ether gave the hydrochloride of β-ADA as very hygroscopic leaflets, mp 115—116°C (uncor.). Found: C, 46.38; H, 9.57; N, 15.51%. Calcd for $C_7H_{17}N_2OCl$: C, 46.53; H, 9.48; N, 15.51%.

0.1 N *Potassium Hydroxide.* Prepared according to Armstrong,[20] standardized against potassium hydrogen phthalate, and stored under a nitrogen atmosphere.

0.01 M *Copper(II) Nitrate.* Prepared by dissolving copper (II) nitrate trihydrate in water and standardized by chelatometric titrations.[21]

All the reagents used were of reagent grade and deionized water was used throughout.

pH Titrations. An aqueous solution of a ligand and copper(II) nitrate (0.002—0.008 M) was titrated with 0.1 N potassium hydroxide under a nitrogen atmosphere at 25°C (μ=0.1(KNO_3)). For the calculation of the acid dissociation constants, the ligands were titrated in the absence of copper(II) nitrate. The pH values were measured with a Radiometer PHM 4d pH meter equipped with a G202C glass electrode and a K401 saturated calomel electrode. The system was standardized with a Horiba and a Radiometer standard buffer solution (pH 4.01 and 6.48). Conversion of pH to $-\log [H^+]$, where $[H^+]$ refers to the hydrogen ion concentration, and determination of the apparent ion product of water pK_w' were made by titrating 0.01 N nitric acid with 0.1 N potassium hydroxide under the same conditions. The values $[H^+]=10^{-pH}/0.85$ and $pK_w'=13.88$ were used in the calculations.

Results and Discussion

Titration Curves. The titration curves of the amides both in the absence and presence of copper(II) are shown in Fig. 1. The curves for the GA-copper (II) systems reveal that two protons are liberated from a ligand in the presence of copper(II), whereas only one proton is liberated in the GDA-copper(II) system. The two protons undoubtedly can be ascribed

5) G. K. Pagenkopf and D. W. Margerum, *J. Amer. Chem. Soc.*, **90**, 6963 (1968).
6) R. F. Pasternack, M. Angwin, and E. Gibbs, *ibid.*, **92**, 5878 (1970).
7) M. K. Kim and A. E. Martell, *Biochemistry*, **3**, 1169 (1964).
8) M. K. Kim and A. E. Martell, *J. Amer. Chem. Soc.*, **88**, 914 (1966).
9) O. Yamauchi, Y. Hirano, Y. Nakao, and A. Nakahara, *Can. J. Chem.*, **47**, 3441 (1969).
10) S. P. Datta and B. R. Rabin, *Trans. Faraday Soc.*, **52**, 1123 (1956).
11) N. C. Li, B. E. Doody, and J. M. White, *J. Amer. Chem. Soc.*, **79**, 5859 (1957).
12) H. Sigel, *Angew. Chem.*, **80**, 124 (1968).
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15) A. J. Speziale and P. C. Hamm, *J. Amer. Chem. Soc.*, **78**, 2558, 5583 (1956).
16) C. Sannie, *Bull. Soc. Chim. Fr.*, **9**, 487 (1942).
17) E. Fischer and E. Fourneau, *Ber.*, **34**, 2868 (1901).
18) Y. Nakao, H. Ishibashi, and A. Nakahara, This Bulletin, **43**, 3457 (1970).
19) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 758 (1935).
20) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases," Methuen and Co., Ltd., London (1962).
21) K. Ueno, "Kireto Tekiteiho," Nankodo Co., Ltd., Tokyo (1960).

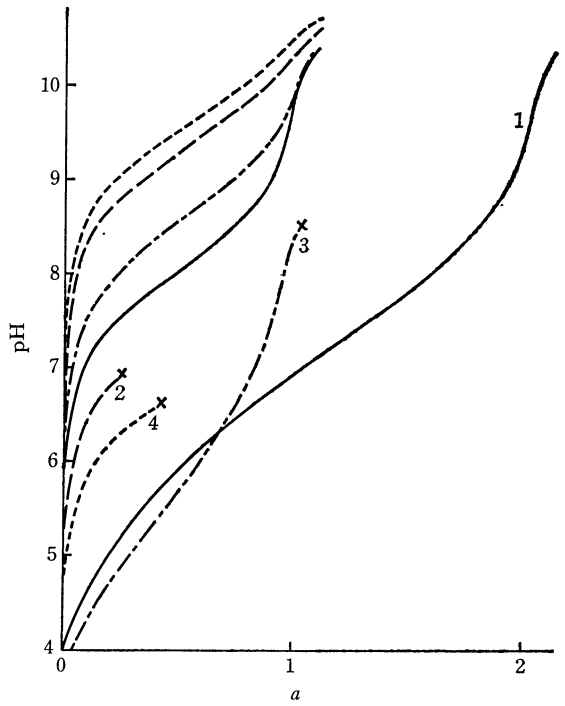


Fig. 1. Titration curves for the amino acid amides in the absence and in the presence of copper(II) ion.
—— GA; ——— β-AA; ·—·—· GDA; ------ β-ADA
Curves 1—4 correspond to the titrations of the following system:
curve 1 GA=0.004182 M; Cu(II)=0.002088 M
curve 2 β-AA=0.004272 M; Cu(II)=0.001044 M
curve 3 GDA=0.003980 M; Cu(II)=0.002046 M
curve 4 β-ADA=0.004000 M; Cu(II)=0.002046 M
a: Moles of alkali added per mole of ligand
×: Precipitate formation

to the dissociation of the ammonium and amide groups of protonated GA. The titrations of the solutions containing copper(II) and the β-alaninamides at various ratios resulted in the precipitations of the hydrolyzed species of copper(II). No sufficient data were obtained. This might indicate that the β-alanine derivatives, in sharp contrast to the glycine derivatives, form unstable complexes with copper(II), where the copper(II)-ligand bonds are susceptible to hydrolytic cleavage.

Calculation of the Equilibrium Constants. We see from the titration curves that the equilibria for the GA-copper(II) systems are somewhat complicated, and the usual Bjerrum method, which is effective for the *N*,*N*-diethylamides, does not seem to be suitable for calculating the constants appearing in these systems. Various computer programs for the non-linear least-squares treatment of data have hitherto been proposed and applied to such complex systems. Recently Sayce[22] reported a general program SCOGS, which evaluates data by this method based on the minimization of the sum of the squares of the residuals in the titer of base, in contrast to the earlier programs which seek to minimize the sum of the squares of the residuals in the analytical hydrogen ion concentrations.

Since the former apparently seems to be easier in the weighing procedure, we also attempted the least-squares refinements as suggested by Sayce. Our program is in principle analogous to SCOGS and earlier programs and consists of the Newton-Raphson iterations, which calculate from the initial estimates of the constants the free ligand and free metal ion concentrations, using the mole balance equations for the total ligand and total metal ion concentrations, the setting up of the matrix for the least-squares treatment, which gives the shifts in the constants by matrix inversion, and the output of the refined constants and their estimated standard deviations. The output also includes the calculated concentration of alkali, the difference of the calculated titer from the experimental, the free ligand and free metal concentrations, and the individual amounts of coordinated species relative to the total metal ion concentration, for each data point. In the present version of the program, partial derivatives were obtained arithmetically for each system, and the calculated shifts in the constants were in the form of the constants and not in their logarithms. The residuals in the concentrations of alkali were calculated from the equation for the electroneutrality of solution. Initial estimates of the constants were obtained by some other methods or taken from literature, and the program accepted up to ten sets of such estimates for a system. The standard deviations of the constants were estimated from the elements of the inverted matrix and the assumed error in the titer of alkali. The subroutine for matrix inversion is conventional and that for least-squares treatment is described elsewhere.[23]

The reaction sequences expected from the titration curves of the 1 : 1 and 2 : 1 GA-copper(II) systems can be expressed by the following equlibria (1) through (5) with the relevant equilibrium constants K_1, K_1^H, K_2, K_{c_1}, and K_{c_2} defined by Eqs. (6) through (10):

$$Cu^{2+} + HL \overset{K_1}{\rightleftharpoons} Cu(HL)^{2+} \quad (1)$$

$$Cu(HL)^{2+} \overset{K_1^H}{\rightleftharpoons} CuL^{+} + H^{+} \quad (2)$$

$$Cu(HL)^{2+} + HL \overset{K_2}{\rightleftharpoons} Cu(HL)_2^{2+} \quad (3)$$

$$Cu(HL)_2^{2+} \overset{K_{c_1}}{\rightleftharpoons} Cu(HL\cdot L)^{+} + H^{+} \quad (4)$$

$$Cu(HL\cdot L)^{+} \overset{K_{c_2}}{\rightleftharpoons} CuL_2 + H^{+} \quad (5)$$

$$K_1 = \frac{[Cu(HL)^{2+}]}{[Cu^{2+}][HL]} \quad (6)$$

$$K_1^H = \frac{[CuL^{+}][H^{+}]}{[Cu(HL)^{2+}]} \quad (7)$$

$$K_2 = \frac{[Cu(HL)_2^{2+}]}{[Cu(HL)^{2+}][HL]} \quad (8)$$

$$K_{c_1} = \frac{[Cu(HL\cdot L)^{+}][H^{+}]}{[Cu(HL)_2^{2+}]} \quad (9)$$

$$K_{c_2} = \frac{[CuL_2][H^{+}]}{[Cu(HL\cdot L)^{+}]} \quad (10)$$

22) I. G. Sayce, *Talanta*, **15**, 1397 (1968).

23) K. B. Wiberg, "Computer Programming for Chemists," W. A. Benjamin, Inc., New York (1965).

TABLE 1. EQUILIBRIUM CONSTANTS (25±0.05°C; μ=0.1 (KNO_3))[a]

Ligand	$pK_{a_{COOH}}$	$pK_{a_{NH_3^+}}$	$\log K_1$	pK_1^H	$\log K_2$	pK_{c_1}	pK_{c_2}
GA[b]		7.96±0.003	5.22±0.005		4.36±0.010	6.95±0.007	8.17±0.004
			5.30±0.002[c]	6.79±0.005[c]			
β-AA[d]		9.23±0.008	5.0_9		4.5		
			5.1_0[e]		4.4[e]		
GDA		8.49±0.005	6.18±0.003		5.12±0.005		
			6.15[e]		5.14[e]		
β-ADA[d]		9.48±0.005	5.51±0.04		5.2_4±0.1		
			5.55[e]		5.1_5[e]		
Gly·Gly	3.14±0.006	8.09±0.006	5.50±0.001			4.10±0.001	
			5.50[f]			4.10[f]	
β-Ala·Gly	3.22±0.009	9.45±0.009	5.45±0.001			4.09±0.001	
			5.45[f]			4.08[f]	
Glycine[g]	2.41	9.63	8.20[e]		6.90[e]		
β-Alanine[g]	3.55	10.15	6.99[e]		5.55[e]		

a) Variances are expressed in standard deviations. For all the data used for calculation, the differences between the observed and calculated titers of 0.1 N potassium hydroxide were within 0.02 ml for the titers ranging from 0 to 2.5 ml.
b) The following values have been reported:
$pK_{a_{NH_3^+}}$=8.04±0.02; $\log K_1$=5.40±0.04; pK_1^H=7.01±0.04 (25°C; μ=0.1 ($NaClO_4$))[12]
$pK_{a_{NH_3^+}}$=8.19±0.01; $\log K_1$=5.4; $\log K_2$=4.4; pK_{c_1}=7.7; pK_{c_2}=8.6 (25°C; μ=1.0 ($NaClO_4$)).[13]
c) Calculated from the data obtained by titrating a 1 : 1 GA-copper(II) system.
d) The $\log K_1$ and $\log K_2$ values are inaccurate owing to precipitation.
e) Calculated from the $\bar{n}$ values.[24]
f) Calculated by the method of Datta and Rabin.[10]
g) Constants included for comparison and are taken from our previous studies[9] and unpublished results.

where HL and L refer to GA and GA deprotonated from the amide nitrogen atom, respectively. Least-squares adjustment taking the equlibria (1), (3), (4), and (5) into account was satisfactory for the 2 : 1 ligand-copper(II) system over the pH range 4—9. Attempts to include the equilibrium (2) were not successful, indicating that this process is negligible if not improbable in this system. Constant K_1^H was obtained from the titration of a 1 : 1 ligand-copper(II) mixture.

For the β-AA-copper(II) system, evaluation of the data may be inaccurate because of the precipitate formation at a very early stage of titration. Considering the pH range of the data and the expected stability of the complex, deprotonation reactions (2), (4), and (5) seemed to be negligible, and only the calculation of the constants K_1 and K_2 for the equilibria (1) and (3) was attempted. On the other hand, the *N,N*-diethylamides have no dissociable amide hydrogens and form complexes of the types CuL^{2+} and CuL_2^{2+}, where L refers to free ligand, according to equlibria similar to (1) and (3). Thus, the stability constants K_1 and K_2 were calculated by the Bjerrum and the least-squares methods. The amide β-ADA was similar in behavior to β-AA and did not afford sufficient data owing to precipitation.

Interpretation of the Equilibrium Constants. The calculated constants for the amides are shown in Table 1 along with the values for Gly·Gly, β-Ala·Gly, glycine, and β-alanine. The dipeptide-copper(II) systems were titrated under the same conditions, and the K_1 and K_{c_1} values, which describe the reactions analogous to (1) and (2), were calculated by the method of Datta and Rabin[10] and by the present method.

The stability constants $\log K_1$ and $\log K_2$ for GDA and β-ADA calculated by the present method agreed with those obtained by the method of Irving and Rossotti[24] from the $\bar{n}$ values (average number of ligand molecules bound to a metal ion) within 0.1 log unit.

Also, the constants $\log K_1$ and $-\log K_c$ (pK_{c_1}) for the dipetides calculated by the present treatment were in excellent agreement with those obtained by the method of Datta and Rabin.

As regards the GA-copper(II) systems, Regardh[13] obtained the four constants K_1, K_2, K_{c_1}, and K_{c_2} according to the method of Bjerrum by titrating a 2 : 1 ligand-copper(II) system at 25°C (μ=1.0($NaClO_4$)). Considering the differences in the conditions and the method of calculation, they are in good agreement with the present values. As Regardh remarked, however, serious deviations were observed between the calculated and the experimental values in the

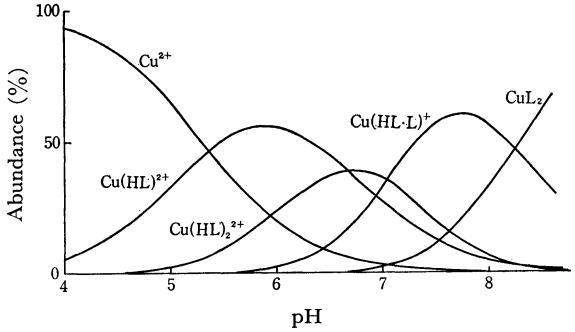


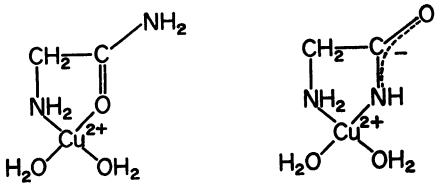
Fig. 2. Abundances of coordinated species in the 2 : 1 GA-copper(II) system; calculated from the constants listed in Table 1.

24) H. Irving and H. S. Rossotti, *J. Chem. Soc.*, **1953**, 3397.

pH region where two or more concomitant reactions take place, because only a limited number of reactions were taken into consideration. According to the least-squares treatment of data, a better fit to the experimental curve could be obtained over the entire pH range in question. The relative amounts of the coordinated species present in a 2 : 1 ligand-copper (II) system at various pH values are shown in Fig. 2. We see that in the pH range 6.2—8.0 at least three of the four species exist in considerable amounts. Constant K_1^H for the equlibrium (2) could not be obtained from the titration of a 2 : 1 ligand-copper(II) system, which indicates the predominance of the formation of the 2 : 1 chelate over that of the deprotonated 1 : 1 chelate.

Stability constants for the β-alaninamides were calculated from a very small number of data as compared with those for the glycinamides and are less reliable. For the β-AA-copper(II) system, the stability constants can be influenced by the probable errors due to hydrolysis reactions.

Komorita *et al.*[25] inferred from the spectral studies that the preferred coordination sites of α-amino acid amides in acid solution are the amino nitrogen and the carbonyl oxygen as shown by structure III and that in alkaline solution the amide nitrogen replaces oxygen (IV). Figure 2 indicates that in the pH range 4—7 the complexes $Cu(HL)^{2+}$ and $Cu(HL)_2^{2+}$ predominate, whereas at higher pH values the amounts of the deprotonated species $Cu(HL\cdot L)^+$ and CuL_2 exceed the amount of $Cu(HL)_2^{2+}$. The finding is consistent with that from the spectral studies.



III IV

When compared with the dipeptide-copper(II) chelates, the 1 : 1 GA-copper(II) chelate exhibits a remarkable difference in the pK_{c_1} value (6.95) which is more than 2.8 log units higher than the values for the dipeptides. Therefore, the deprotonated chelates CuL of Gly·Gly and β-Ala·Gly predominate over $Cu(HL)^+$ in wide pH ranges as demonstrated in Figs. 3 and 4. The fact probably points to the difference in stability of the deprotonated chelates.

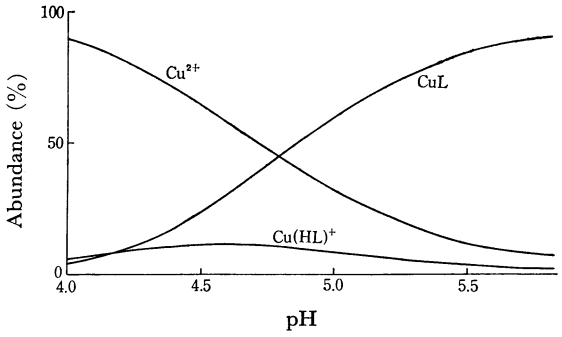


Fig. 3. Abundances of coordinated species in the 1 : 1 Gly·Gly-copper(II) system; calculated from the constants listed in Table 1. Similar curves were reported by Kim and Martell.[7]

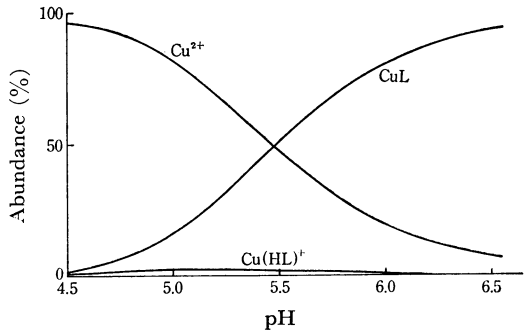


Fig. 4. Abundances of coordinated species in the 1 : 1 β-Ala·Gly-copper(II) system; calculated from the constants listed in Table 1.

Stability constants log K_1 for the glycinamides are now found to be greater than those for the corresponding β-alaninamides. Although the values for β-AA may not be reliable, the hydrolysis reactions observed during the titrations manifest that β-AA-copper(II) chelates are less stable than the GA-copper-(II) chelates. On the other hand, there is practically no difference between the log K_1 values of the Gly·Gly-and β-Ala·Gly-copper(II) chelates in spite of the large difference between those of the glycine- and β-alanine-copper(II) chelates. We explained the fact as due to the formation of the fused-ring structures, IIa and IIb,[9] where the steric requirements of the individual rings might be cancelled or dispersed over the chelate system as a whole to give chelates of equal stability. The present results indicate that the ring size produces a decisive effect on the chelate stability when the substituents on the amide nitrogen do not take part in chelation. Hence, the equal stability of the Gly·Gly- and β-Ala·Gly-copper(II) chelates appears to be better explained by the structures IIa and IIb rather than by Ia and Ib. As suggested by Tanaka *et al.*,[26] there seems to be a linear relationship between the differences in the pK_a values of the amino groups of the glycine derivatives and of the corresponding β-alanine derivatives investigated and the differences in the log K_1 values. Although the small difference observed for the dipeptides can be explained according to this relationship by the Rabin models Ia and Ib as well, we are more inclined to favor the fused-ring structures for the charged dipeptide-copper(II) chelates formed in acid solution, from the findings described above. Very recently Barnet *et al.*[27] determined the crystal structures of cobalt(III) complexes of Gly·Gly, of which bis(glycylglycinato)cobalt(III) perchlorate was shown to have

25) T. Komorita, J. Hidaka, and Y. Shimura, This Bulletin, **42**, 168 (1969).

26) H. Tanaka, A. Yokoyama, Y. Sugiura, and K. Aiba, private communication, 1970.

27) M. T. Barnet, H. C. Freeman, D. A. Buckingham, I-N. Hsu, and D. v. d. Helm, *J. Chem. Soc., D*, **1970**, 367.

two Gly·Gly ions coordinating to cobalt(III) through the amino nitrogen, peptide nitrogen, and carboxyl oxygen with the hydrogen of the peptide group attached to the peptide oxygen. This seems to strongly support our view.

The situation would be somewhat different in the longer peptides, because the carboxyl group is quite apart from the strongly coordinating amino residue, so that it would be rather difficult to arrange the intervening peptide nitrogens around the coordination sphere of copper(II) without dissociation of the peptide hydrogens.

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