Stability of Fused Rings in Metal Chelates. XI. Stability of Dipeptide Amide-Copper(II) Complexes

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The structure-stability relationship between the copper(II) complexes of several dipeptide amides consisting of glycine and/or β-alanine has been investigated by potentiometric titration. The amides of glycylglycine, glycyl-β-alanine, and β-alanylglycine (abbreviated as H_2L) have been found to form complexes of the type $Cu(H_2L)^{2+}$ with log K_1 values 4.80—5.22. The $-\log K_{c_1}$ values for deprotonation of the peptide group are 5.05—5.42 and comparable to those of tripeptides, whereas the $-\log K_{c_2}$ values for the terminal amide group are 7.96 for glycylglycine amide and 8.99 for glycyl-β-alanine amide. This indicates that, at neutral pH, the $-CONH_2$ group coordinates to copper(II) mainly through the carbonyl oxygen. β-Alanyl-β-alanine amide-copper(II) system gave precipitates at an early stage of titration. The structures and relative stability of the complexes of types $Cu(H_2L)^{2+}$, $Cu(HL)^+$, and CuL have been discussed from comparative studies of their stability constants.

Amide groups in amino acid amides and peptides exhibit interesting reactivity toward copper(II) ions. Their modes of reaction reflect the influence of the environment. The reactivity, expressed in terms of the dissociation constant for the amide group in the presence of copper(II), appears to depend on whether the group is in a simple amino acid amide or in a peptide chain and how it can have access to the central metal ion.[1,2]

With this in mind, we have investigated the structure-stability relationship between the copper(II) complexes of several dipeptide amides, in order to get information on the factors affecting the complexing abilities of the amide groups and the probable structures of the resulting complexes in aqueous solution.

For closer comparison with the reported peptide-copper(II) systems,[2,3] the solution equilibria of the copper(II) complexes of glycylglycine amide (abbreviated as Gly·Gly·NH_2), glycyl-β-alanine amide (Gly·β-Ala·NH_2), β-alanylglycine amide (β-Ala·Gly·NH_2), and β-alanyl-β-alanine amide (β-Ala·β-Ala·NH_2) have been studied by potentiometric titration, the results of which are given in the following.

Experimental

All the melting points are uncorrected.

Preparation of Dipeptide Amides and Related Compounds The ethyl ester hydrochlorides of glycine, β-alanine, glycylglycine, and glycyl-β-alanine were prepared according to the ordinary method.[4]

Carbobenzoxyglycylglycine Ethyl Ester. To a solution of 10 g of glycylglycine ethyl ester hydrochloride in 40 ml of water were gradually added 12 g of potassium bicarbonate and then 90 ml of chloroform. Into the resulting mixture was added 8.5 ml of carbobenzoxy chloride over a period of 15—20 min under constant stirring. After it had been stirred at room temperature for 1—2 hr, unreacted carbobenzoxy chloride was treated with pyridine. The organic fraction was washed successively with 2—3% aqueous sodium bicarbonate, dilute hydrochloric acid, and water, and then dried over anhydrous magnesium sulfate. The filtrate was concentrated *in vacuo* to give a crystalline product, which was recrystallized from ethyl acetate–petroleum benzin.

Carbobenzoxyglycyl-β-alanine Ethyl Ester. This was prepared by a similar procedure to that for the carbobenzoxyglycylglycine ethyl ester.

The two materials described above were identified by comparing their melting points with those in the literature.[5,6]

Carbobenzoxy-β-alanylglycine Ethyl Ester. This was prepared in the same manner as reported by Winitz *et al.*[7] To a mixture of 15.6 g of carbobenzoxy-β-alanine[8] prepared from β-alanine and carbobenzoxy chloride, 9.8 g of glycine ethyl ester hydrochloride, and 9.8 ml of triethylamine in 160 ml of chloroform was added 14.5 g of *N,N'*-dicyclohexylcarbodiimide, and the reaction mixture was stirred at room temperature overnight. After removal of *N,N'*-dicyclohexylurea, the filtrate was washed successively with water, dilute hydrochloric acid, saturated aqueous sodium bicarbonate and water, dried over anhydrous magnesium sulfate and evaporated to dryness *in vacuo*. The product was identified by its melting point.[6]

Carbobenzoxy-β-alanyl-β-alanine Ethyl Ester. This was prepared from carbobenzoxy-β-alanine and β-alanine ethyl ester hydrochloride in the same way as for carbobenzoxy-β-alanylglycine ethyl ester.

Carbobenzoxy Derivatives of Glycylglycine-, Glycyl-β-alanine-, β-Alanylglycine-, and β-Alanyl-β-alanine-amide. These compounds were prepared according to the known method.[6] The first three compounds were confirmed by their melting points,[6,9] and elemental analysis. Carbobenzoxy-β-alanyl-β-alanine amide recrystallized from methanol–ether melted at 192—195 °C. Found: C, 57.49; H, 6.49; N, 14.24%. Calcd for $C_{14}H_{19}N_3O_4$: C, 57.32; H, 6.54; N, 14.33%.

Hydrochlorides of Glycylglycine-, Glycyl-β-alanine-, β-Alanylglycine-, and β-Alanyl-β-alanine-amide. These were obtained by the catalytic reduction of the corresponding amide

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2) O. Yamauchi, Y. Nakao, and A. Nakahara, *ibid.*, **46**, 2119 (1973).

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

4) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 2, John Wiley & Sons, Inc., New York (1961), p. 926.

5) R. W. Young, K. H. Wood, R. J. Joyce, and G. W. Anderson, *J. Amer. Chem. Soc.*, **78**, 2126 (1956).

6) H. T. Hanson and E. L. Smith, *J. Biol. Chem.*, **175**, 833 (1948).

7) M. Winitz, L. B. Frankenthal, N. Izumiya, S. M. Birnbaum, C. G. Baker, and J. P. Greenstein, *J. Amer. Chem. Soc.*, **78**, 2423 (1956).

8) Y. Nakao, H. Ishibashi, and A. Nakahara, This Bulletin, **43**, 3457 (1970).

9) J. S. Fruton and M. J. Bergmann, *J. Biol. Chem.*, **145**, 253 (1942).

derivatives of the carbobenzoxy peptides by using palladium black as a catalyst. In all cases, methanol was used as solvent with a small amount of hydrochloric acid. Glycylglycine amide hydrochloride and glycyl-β-alanine amide hydrochloride were recrystallized from methanol–ethyl acetate, whereas β-alanylglycine amide hydrochloride and β-alanyl-β-alanine amide hydrochloride were recrystallized from methanol–ether.

Glycylglycine Amide Hydrochloride: Mp 192—193 °C. Found: C, 28.53; H, 6.27; N, 25.46%. Calcd for $C_4H_{10}N_3O_2Cl$: C, 28.66; H, 6.03; N, 25.07%.

Glycyl-β-alanine Amide Hydrochloride: Mp 166—168 °C. Found: C, 33.05; H, 6.53; N, 23.25%. Calcd for $C_5H_{12}N_3O_2Cl$: C, 33.06; H, 6.67; N, 23.14%.

β-Alanylglycine Amide Hydrochloride: Mp 182—183 °C. Found: C, 32.98; H, 6.59; N, 23.58%. Calcd for $C_5H_{12}N_3O_2Cl$: C, 33.06; H, 6.67; N, 23.14%.

β-Alanyl-β-alanine Amide Hydrochloride: Mp 135—137 °C. Found: C, 36.93; H, 7.05; N, 21.48%. Calcd for $C_6H_{14}N_3O_2Cl$: C, 36.83; H, 7.23; N, 21.48%.

Glycylglycine Amide Acetate: This was prepared according to the method of Fruton and Bergmann.[9]

Preparation of the Neutral Copper(II) Complex of Glycylglycine Amide To a solution of 0.75 g of glycylglycine amide acetate in 10 ml of water was added freshly precipitated copper(II) hydroxide obtained from 1.5 g of copper(II) sulfate pentahydrate. The mixture was stirred at room temperature for about 30 min, and, after removal of excessive copper(II) hydroxide, added into ethanol–ether (2 : 1 by volume) under vigorous stirring. The complex was obtained as violet crystals. Found: C, 22.35; H, 4.53; N, 18.67%. Calcd for $[Cu(C_4H_7N_3O_2)(OH_2)]\cdot 1/2H_2O$: C, 21.87; H, 4.60; N, 19.13%.

pH Titration. Aqueous solutions (4.0×10^{-3} M; μ= 0.1 (KNO_3)) of the dipeptide amides were titrated in the absence and the presence of an equimolar amount of copper(II) nitrate.

The apparatus and the procedure were the same as reported previously.[2]

Results and Discussion

Titration Curves and Equilibrium Constants. The pH titration curves of the dipeptide amides in the absence and the presence of copper(II) nitrate are shown in Fig. 1. The amides with the β-alanyl group at the NH_2-terminus gave precipitates during the titration in the presence of copper(II). Since the structures of the dipeptide amides are closely related to those of the reported di- and tri-peptides[2,3] and the behavior of the terminal amide group is considered to be very similar to that of simple amino acid amides,[1] it is reasonable to assume from the titration curves the following reaction sequences and the species involved therein:

$$Cu^{2+} + H_2L \overset{K_1}{\rightleftharpoons} Cu(H_2L)^{2+} \quad (1)$$

$$Cu(H_2L)^{2+} \overset{K_{c_1}}{\rightleftharpoons} Cu(HL)^{+} + H^{+} \quad (2)$$

$$Cu(HL)^{+} \overset{K_{c_2}}{\rightleftharpoons} CuL + H^{+} \quad (3)$$

where H_2L refers to free dipeptide amide, and $Cu(HL)^+$ and CuL to the complexes formed upon removal of one and two protons, respectively, from the peptide linkages in the dipositive complex $Cu(H_2L)^{2+}$. The

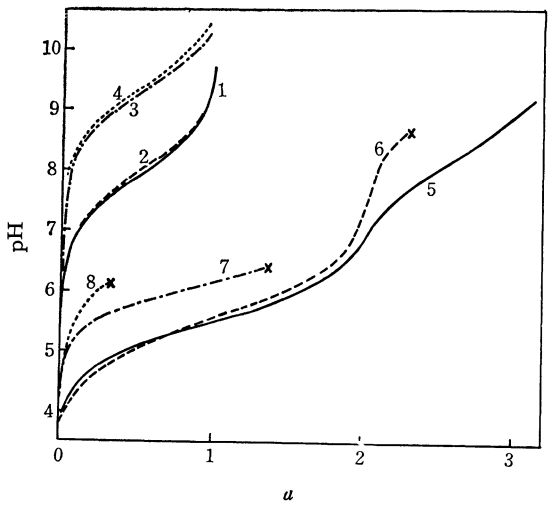


Fig. 1. Titration curves for the dipeptide amides in the absence and the presence of copper(II).
Curves 1—4: ligand alone
Curves 5—8: ligand : Cu(II) = 1 : 1
—— Gly·Gly·NH_2; ---- Gly·β-Ala·NH_2;
–––– β-Ala·Gly·NH_2; ····· β-Ala·β-Ala·NH_2
a: Moles of KOH added per mole of ligand.
x: Precipitation occurred at this point.

relevant equilibrium constants are defined by the following equations.

$$K_1 = \frac{[Cu(H_2L)^{2+}]}{[Cu^{2+}][H_2L]} \quad (4)$$

$$K_{c_1} = \frac{[Cu(HL)^{+}][H^{+}]}{[Cu(H_2L)^{2+}]} \quad (5)$$

$$K_{c_2} = \frac{[CuL][H^{+}]}{[Cu(HL)^{+}]} \quad (6)$$

The curve for Gly·Gly·NH_2–copper(II) indicates the dissociation of the third proton from the water molecule in the complex at high pH to give a hydroxy complex $CuL(OH)^-$ according to the equation:

$$CuL \overset{K_{OH}}{\rightleftharpoons} CuL(OH)^{-} + H^{+} \quad (7)$$

where

$$K_{OH} = \frac{[CuL(OH)^{-}][H^{+}]}{[CuL]} \quad (8)$$

Calculation of the equilibrium constants K_1, K_{c_1}, K_{c_2} and K_{OH} was carried out by the method of non-linear least-squares with the use of a NEAC 2200/500 computer. Derivation of the equations for the least-squares treatment and the computer program were essentially the same as those reported for the tripeptide-copper(II) systems.[2] Because of precipitation, the K_{c_2} value for β-Ala·Gly·NH_2 and all the constants for β-Ala·β-Ala·NH_2 could not be calculated.

The calculated equilibrium constants are shown in Table 1 together with the corresponding constants for the amino acid amide-,[1] dipeptide-,[3] and tripeptide-copper(II)[2] systems included for comparison. The reliability of the calculated constants was confirmed by duplicate or triplicate titration and by comparing the theoretical curves with the experimental ones, the deviations expressed in titer being negligibly small

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

Ligand[b]	pK_a(COOH)	pK_a(NH_3^+)	log K_1	pK_{c_1}	pK_{c_2}	pK_{OH}	log $K_1K_{c_1}$	log $K_1K_{c_1}K_{c_2}$
Gly·Gly·NH_2		7.81±0.01	4.80±0.02	5.05±0.02	7.96±0.01	9.77±0.01	−0.25	−0.21
Gly·β-Ala·NH_2		7.87±0.03	5.22±0.01	5.42±0.01	8.99±0.01	c)	−0.20	−9.19
β-Ala·Gly·NH		9.18±0.03	5.16±0.03	5.39±0.04	c)	c)	−0.23	
β-Ala·β-Ala·NH_2		9.25±0.06	c)	c)	c)	c)		
Gly·Gly·Gly[d]	3.26±0.01	7.93±0.03	5.25±0.01	5.23±0.01	6.73±0.01		0.02	−6.71
Gly·β-Ala·Gly[d]	3.34±0.02	8.09±0.01	5.60±0.01	5.36±0.01	5.74±0.01		0.24	−5.50
Gly·Gly[e]	3.14±0.02	8.09±0.02	5.50±0.01	4.10±0.01			1.40	
Gly·β-Ala[f]	3.98	8.16	5.70±0.09	4.64±0.06			1.06	
Glycinamide[e]		7.96±0.01	5.30±0.01	6.79±0.02			−1.49	
β-Alaninamide[e]		9.23±0.02	5.1					

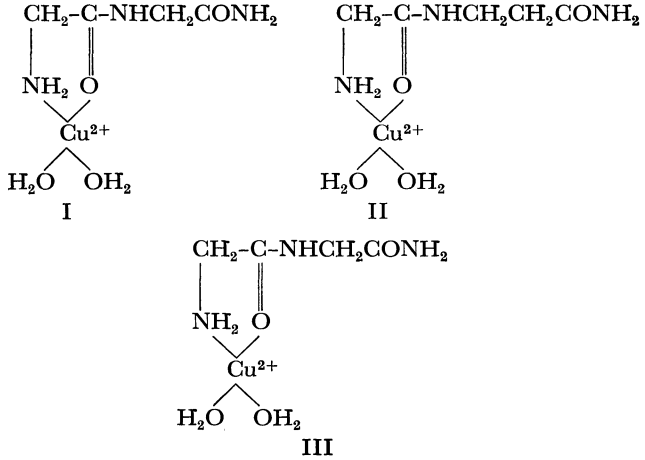
a) Variances are three times the estimated standard deviations. b) Abbreviations used: Gly·Gly·Gly=glycylglycylglycine; Gly·β-Ala·Gly=glycyl-β-alanine. c) Not determined because of precipitation. d) Ref. 2. e) Ref. 1. f) Ref. 3.

(less than 0.03 ml for the titers of 0—3.0 ml) for all the systems in the pH ranges studied.

The log K_1 values are 4.80—5.22 and close to those for the tripeptide-copper(II) systems. A possible reason for the low value for Gly·Gly·NH_2 might be that this ligand has the lowest pK_a value. This system has been reported recently by Sigel *et al.*[10] who obtained the value of 5.05 for log K_1.

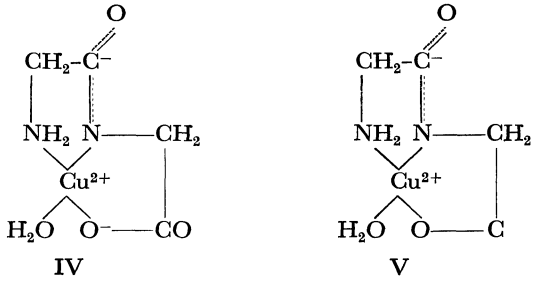
Dissociation of the peptide hydrogens in the complexed species are measured by means of pK_{c_1} (−log K_{c_1}) and pK_{c_2} (−log K_{c_2}), which can be compared with the corresponding values for the dipeptides and the tripeptides. The pK_{c_1} values are essentially the same for the three amides and seem to be independent of the nature of the *N*-terminal amino acid, whereas the pK_{c_2} values reflect the steric requirements around copper(II) and are much higher than those for the tripeptides. The reported values[10] of pK_{c_2} (7.29) and estimated pK_{OH} (−log K_{OH}) (~8.3) for Gly·Gly·NH_2 differ considerably from the present values, this being ascribable to precipitation which was not observed in our experiment.

Structure-Stability Relationship. The dipeptide amides in acid solution are most probably bidentate ligands like glycinamide and tripeptides, coordinating through the amino and the peptide carbonyl group as shown by the following structures I—III.[1,2,11,12]



That the log K_1 value for β-Ala·Gly·NH_2 is close to that for Gly·β-Ala·NH_2 in spite of the higher pK_a may reasonably be explained by the less stable six-membered ring (III) as compared with the five-membered rings in I and II. These structures for $Cu(H_2L)^{2+}$ may be accepted, because the pK_a and log K_1 values are in excellent agreement with the corresponding values[1] for glycinamide and β-alaninamide both of which are known to form chelates in acid solution through the amino nitrogen and the amide oxygen.[1,12]

Deprotonation from the peptide linkage leads to $Cu(HL)^+$, where the pK_{c_1} values are comparable to those of tripeptides, but about 1 log unit higher than those of dipeptides. This may be interpreted in terms of the more effective coordination by the carboxyl group in dipeptides (IV) than the amide carbonyl group in dipeptide amides (V) and tripeptides.



The degrees of formation of $Cu(HL)^+$ at a fixed pH can be expressed by the log $K_1K_{c_1}$ values, which are found to be around −0.2 as compared with 0—0.25 and 1—1.4 for the structurally related tripeptide-copper(II) and the dipeptide-copper(II) systems, respetively. As far as these peptide-copper(II) complexes are concerned, the 5-6-membered fused-ring systems are as stable as the 5-5-membered when compared on the basis of log $K_1K_{c_1}$. It is interesting to note in this connection that Weatherburn *et al.*[13] reported a similar stability relationship between the nickel(II)

10) H. Sigel, R. Griesser, and B. Prijs, *Z. Naturforsch.*, **27b**, 353 (1972).

11) H. C. Freeman, "The Biochemistry of Copper," ed. by J. Peisach, P. Aisen, and W. E. Blumberg, Academic Press, Inc., New York (1966), p. 87.

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

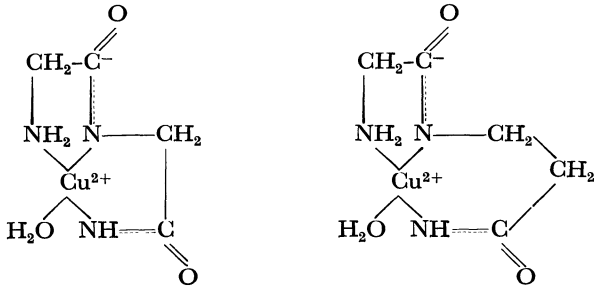
13) D. C. Weatherburn, E. J. Billo, J. P. Jones, and D. W. Margerum, *Inorg. Chem.*, **9**, 1557 (1970).

complexes of diethylenetriamine (5-5-membered) and *N*-(2-aminoethyl)-1,3-propanediamine (5-6-membered).

In dilute aqueous solution, the complexes of type CuL formed upon dissociation of the second proton from $Cu(HL)^+$ may be described by rigid fused-ring structures like VI and VII, whose relative stability is inferred from the log $K_1K_{c_1}K_{c_2}$ values.

$\log K_1K_{c_1}K_{c_2}$

−8.21 −9.19



VI VII

The stability-determining factor is definitely the pK_{c_2} value, which is low for Gly·Gly·NH_2 (7.96) and high for Gly·β-Ala·NH_2 (8.99). Thus, Gly·Gly·NH_2-Cu(II) (VI) is estimated to be more stable than Gly·β-Ala·NH_2-Cu(II) (VII), and the stability order is the reverse of that found for the Schiff base-copper(II) complexes[14] and reminds us of that between glycylglycine- and glycyl-β-alanine-copper(II), although the stability difference is much smaller. The complexes of β-Ala·Gly·NH_2 and β-Ala·NH_2 are supposed to be less stable because they were hydrolyzed to form precipitates at pH below 6.4.

When compared with the tripeptides, the dipeptide amides lose a proton from the terminal amide group at higher pH. A reasonable explanation for this may be that deprotonation from the peptide group nearest the COOH-terminus of tripeptides results in the formation of an additional chelate ring which is considered to increase the chelate stability by the "chelate effect," whereas the deprotonation from the dipeptide amides does not affect the stability in the same way.

The abundance of the coordinated species in the Gly·Gly·NH_2- and Gly·β-Ala·NH_2-copper(II) systems is shown in Figs. 2 and 3, respectively. One important feature of the curves is that the most abundant species at neutral pH is $Cu(HL)^+$ as described by V where the amide NH_2 group remains intact. This suggests that, at physiological pH, $-CONH_2$ groups from the asparagine or the glutamine residue in peptide chains coordinate to copper(II), if at all, through their carbonyl oxygen and not through the nitrogen as is usually the case with oligopeptides.

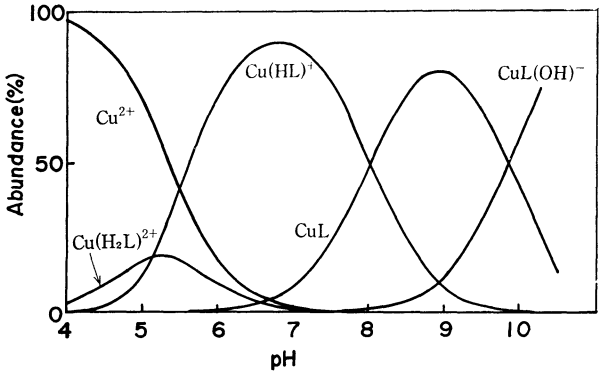


Fig. 2. Calculated abundances of the coordinated species in the 1 : 1 Gly·Gly·NH_2-Cu(II) system.

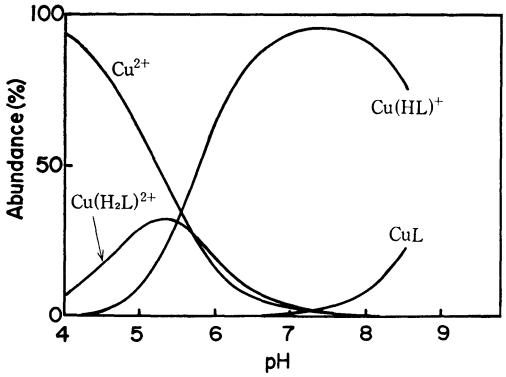


Fig. 3. Calculated abundances of the coordinated species in the 1 : 1 Gly·β-Ala·NH_2-Cu(II) system.

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14) A. Nakahara, H. Yamamoto, and H. Matsumoto, This Bulletin, **37**, 1137 (1964).