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# Stability of Binary and Ternary β-Alanine Containing Dipeptide Copper(II) Complexes [1]

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*Equilibrium constants have been measured by potentiometric pH titration for protonation and $Cu^{2+}$ coordination of glycinamide, glycylglycine, glycyl-β-alanine, β-alanylglycine, β-alanyl-β-alanine, and β-alaninamide. Besides the binary complexes $CuL^+$ and Cu(L–H) the mixed-ligand complexes with 2,2'-bipyridyl, Cu(Bpy)$L^+$ and Cu(Bpy)(L–H), were also studied. The species CuL and Cu(Bpy)L are formed with all the mentioned ligands, while ionization of the amide nitrogen and its coordination occurs in the binary systems only with the first four ligands and in the ternary systems only with the first three; in all the other systems hydroxocomplex formation appears to be favored over amide ionization. When allowance is made for amino group basicity, the order of decreasing stabilities for the $Cu^{2+}$–dipeptide complexes is Gly-gly > Gly-β-ala > β-Ala-gly > β-Ala-β-ala. Analysis of other linked chelate ring systems such as multidentate amines, with several metal ions, reveals that when amino group basicity is considered, chelates containing linked 5-membered rings are favored over systems with 6-membered rings when there are 2 or fewer trigonal atoms in the rings.*

## Introduction

β-Alanine is a component of pantothenic acid, as well as of the peptides carnosine and anserine; it is also among the end products of purine metabolism. This naturally occurring amino acid forms 6-membered chelates which are somewhat less stable than the 5-membered chelates of glycinate, an observation which holds also for the corresponding ternary $Cu^{2+}$ complexes with 2,2'-bipyridyl (Bpy) [2].

Complex formation between $Cu^{2+}$ and peptides in aqueous solution starts with the terminal amino group and not from the carboxylate end. In order to learn how coordination may be altered by the kind of amino acids in the two terminal residues of peptides or proteins, we have been studying the simplest models, *i.e.* dipeptides; they contain all of the binding sites of interest. The first aim was to evaluate the influence of bulky alkyl side chains on the stability and acidity of complexes formed in binary $Cu^{2+}$/dipeptide and ternary 2,2'-bipyridyl/$Cu^{2+}$/dipeptide systems [3]; the next was to study the influence of alkyl side chains containing hydroxy or thioether groups [4]. Now, we are dealing with the binary and ternary $Cu^{2+}$ complexes of dipeptides (HL) containing glycinate and/or β-alaninate, thus allowing us to evaluate the influence of 5/5, 5/6 and 6/6 rings on the stability of these complex species. Glycinamide and β-alaninamide were included for comparison.

## Experimental

Glycyl-β-alanine was purchased from Fluka AG, Buchs, Switzerland; β-alanylglycine was from Sigma Chemical Co., St. Louis, Mo., U.S.A., and β-alanyl-β-alanine from Cyclo Chemical, Division Travenol Laboratories Inc., Los Angeles, Calif., U.S.A. The hydrochloride of β-alaninamide was synthesized as described by Rapport *et al.* [5]; the melting point agreed with the one given in the literature and the elemental analysis was also satisfactory: Calcd. for $C_3H_9N_2OCl$: C, 28.93; H, 7.28; N, 22.49%. Found: C, 28.91; H, 7.25; N, 22.49%. All the other reagents were the same as used earlier [3, 4].

The potentiometric pH-titrations ($I$ = 0.1, $NaClO_4$; 25 °C) were performed under $N_2$ as described previously [3, 6]. The acidity constants ($K^H_{H_2L}$ and $K^H_{HL}$) of the dipeptides and the stability and acidity constants of the binary ($K^{Cu}_{CuL}$ and $K^H_{CuL}$) and ternary complexes ($K^{Cu(Bpy)}_{Cu(Bpy)L}$ and $K^H_{Cu(Bpy)L}$) were determined exactly (including concentrations and numbers of titrations) as before [3]. The pH range

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

| HL | $pK^{H}_{H_2L}$ | $pK^{H}_{HL}$ | $pK^{2H}_{(Cu+H_2L)}$ | $pK^{3H}_{(Cu+H_2L)}$ |
|---|---|---|---|---|
| H(glycinamide)$^+$ (*cf.* [7]) | | 8.04 | | |
| Glycylglycine [3] | 3.11 ± 0.01 | 8.15 ± 0.01 | 5.71 ± 0.07 | 9.70 ± 0.03 |
| Glycyl-β-alanine | 4.08 ± 0.01 | 8.16 ± 0.01 | 6.50 ± 0.06 | 11.17 ± 0.03 |
| β-Alanylglycine | 3.28 ± 0.01 | 9.58 ± 0.01 | 6.71 ± 0.07 | 11.46 ± 0.05 |
| β-Alanyl-β-alanine | 4.13 ± 0.01 | 9.54 ± 0.01 | 7.91 ± 0.07 | (14.73 ± 0.09)[b] |
| H(β-alaninamide)$^+$ | | 9.32 ± 0.02 | | |

[a]The errors given are three times the standard error of the mean value or the sum of the probable systematic errors, whichever is the larger. [b]It appears that the third proton liberated is not due to the ionization of the amide group, but rather to the formation of hydroxo complexes (see also Fn. d of Table II).

for evaluation was such that hydroxo complexes could be ignored, and this was checked by separate titrations [3], especially in those cases were an excess of $Cu^{2+}$ or $Cu^{2+}$/Bpy was used.

## Results and Discussion

The equilibrium constants listed in Tables I and II are defined by eqns. 1–10.

$$H_2L^+ \rightleftharpoons HL + H^+$$

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

$$HL \rightleftharpoons L^- + H^+$$

$$K^{H}_{HL} = [L]\,[H]/[HL] \quad (2)$$

$$Cu^{2+} + H_2L^+ \rightleftharpoons CuL^+ + 2H^+$$

$$K^{2H}_{(Cu+H_2L)} = [CuL]\,[H]^2/([Cu]\,[H_2L]) \quad (3)$$

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

$$K^{3H}_{(Cu+H_2L)} = [Cu(L{-}H)]\,[H]^3/([Cu]\,[H_2L]) \quad (4)$$

$$Cu^{2+} + L^- \rightleftharpoons CuL^+$$

$$K^{Cu}_{CuL} = [CuL]/([Cu]\,[L]) \quad (5)$$

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

$$K^{H}_{CuL} = [H]\,[Cu(L{-}H)]/[CuL] \quad (6)$$

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

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

$$Cu(Bpy)^{2+} + L^- \rightleftharpoons Cu(Bpy)L^+$$

$$K^{Cu(Bpy)}_{Cu(Bpy)L} = [Cu(Bpy)L]/([Cu(Bpy)]\,[L]) \quad (9)$$

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

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

The relative stability of the ternary complexes is best quantified [2] by $\Delta\log K_{Cu}$ (eqn. 11; *cf.* Table II), which is also the logarithm of the equilibrium constant of eqn. 12.

$$\Delta \log K_{Cu} = \log K^{Cu(Bpy)}_{Cu(Bpy)L} - \log K^{Cu}_{CuL}$$

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

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

The equilibrium constants determined for the binary $Cu^{2+}$ complexes with glycyl-β-alaninate (Table II) agree well with earlier results [9, 10], while part of the other constants of the binary systems which have also been determined earlier [9, 11], differ by up to 0.5 log units. However, the values for log $(K^{Cu}_{CuL} \cdot K^{H}_{CuL})$ from the literature [9, 11] agree well with those determined in this work.*

*Stability and Structure of the Complexes*

In the binary complex $CuL^+$ the metal ion is coordinated *via* the terminal amino group and the carbonyl oxygen of the neighboring amide moiety [2–4, 6, 10]. The stabilities of $CuL^+$ for the glycyl-derivatives (*i.e.* glycinamide, glycylglycinate and glycyl-β-alaninate) are quite similar (Table II), as expected [6], which is in accordance with the quite similar basicity of the terminal amino group in these

*For a possible explanation of this observation see the first part of the results section in ref. [12].

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 °C).[a]

| L | $\log K^{Cu}_{CuL}$ | $pK^{H}_{CuL}$ | $\log K^{Cu(Bpy)}_{Cu(Bpy)L}$ | $pK^{H}_{Cu(Bpy)L}$ | $\Delta \log K_{Cu}$ |
|---|---|---|---|---|---|
| Glycinamide [7] | 5.40 | 7.01 | 5.01 | 7.71 | −0.39 |
| Glycylglycinate [3] | 5.55 ± 0.07 | 3.99 ± 0.06 | 5.09 ± 0.10 | 7.77 ± 0.04 | −0.46 |
| Glycyl-β-alaninate | 5.74 ± 0.06 | 4.67 ± 0.08 | 5.39 ± 0.04 | 8.48 ± 0.03 | −0.35 |
| β-Alanylglycinate | 6.15 ± 0.07 | 4.75 ± 0.06 | ~5.0[b] | (~7.0)[c] | |
| β-Alanyl-β-alaninate | 5.76 ± 0.07 | (6.82 ± 0.15)[d] | 5.22 ± 0.04 | (~6.8)[c] | −0.54 |
| β-Alaninamide | 5.63 ± 0.06 | (6.78 ± 0.06)[d] | 5.25 ± 0.10 | (~6.9)[c] | −0.38 |

[a]The errors given are three times the standard error of the mean value. For the constants of the binary $Cu^{2+}$/Bpy system see Ref. [8]. [b]This value is only an estimate because the concentration of $Cu(Bpy)L^{+}$ is very low under the experimental conditions [3]. [c]This value is only an *apparent* constant which refers to 1:1:1 conditions of the reactants ($8 \times 10^{-4}$ *M*). There appears to be no ionization of the amide proton; probably are $Cu(Bpy)^{2+}$–hydroxo complexes formed, which seem also responsible for the occurrence of a precipitate at pH about 7. [d]This value is only an *apparent* constant which refers to 1:1 conditions of the reactants ($8 \times 10^{-4}$ *M*). It appears that the amide proton is not ionized and that rather hydroxo complexes are formed (different reactant ratios give different results), which seem also responsible for the occurrence of a precipitate at pH about 6.8.

ligands (Table I). The same observation holds to a first approximation also for the β-alanyl-derivatives. However, a comparison of the stabilities of $CuL^{+}$ for the glycylderivatives with the corresponding β-alanylderivatives shows that the complexes of the latter ligands are only slightly more stable (Table II) even though the basicity of the β-alanyl-derivatives is larger by about 1.4 log units (Table I). Hence, this result confirms earlier observations [2, 13] that 6-membered chelates are less stable than 5-membered chelates.

The overall picture described for the binary $CuL^{+}$ complexes holds also for the ternary $Cu(Bpy)L^{+}$ complexes. This is expected as in both these complexes the ligands coordinate in a bidentate fashion. Moreover, the values of $\Delta \log K_{Cu}$ (Table II) are in the order expected for the coordination of an N/O donor to $Cu(Bpy)^{2+}$ [2, 6, 14, 15].

The formation of the complexes Cu(L–H) is connected with the deprotonation of the amide group and a structural alteration leading to the coordination of the amide nitrogen. This reaction occurs with all glycylderivatives, though the ionization is somewhat less favored with glycyl-β-alaninate than with glycylglycinate. This is expected because the deprotonation is facilitated by the additional coordination of the carboxylate group of the dipeptide in Cu(L–H) which results in a 6-membered chelate ring only with glycyl-β-alaninate. From the β-alanylderivatives only β-alanylglycinate forms the amide ionized species Cu(L–H), while with the two other ligands hydroxo complexes form, *i.e.* the released proton has its origin in a coordinated water molecule*. This conclusion is confirmed by the identical properties of the β-alanyl-β-alaninate and β-alaninamide systems (Table II); in case of an amide deprotonation the complex of β-alanyl-β-alaninate should be favored.

In the ternary systems deprotonation of the amide moiety occurs only with the glycylderivatives, while all complexes of the β-alanyl-derivatives undergo hydrolysis (Table II). The somewhat greater value of $pK^{H}_{Cu(Bpy)L}$ for glycyl-β-alaninate, compared with glycylglycinate, indicates that there is also some participation of the carboxylate group in (Cu(Bpy)(L–H): there is probably an *intra*-molecular equilibrium between a square-planar [2, 4, 6] and a pentacoordinated [16] $Cu^{2+}$ species; in the latter species all three binding sites of the dipeptide as well as the two nitrogens of 2,2'-bipyridyl may be coordinated.

*Influence of the Ring Size in Linked Chelate Rings*

The relative stabilities of the different sized linked ring systems in the binary dipeptide complexes with reference to free ligand in neutral solutions may be estimated by considering the equilibrium constant for reaction 13.

$$Cu^{2+} + HL \rightleftharpoons Cu(L{-}H) + 2H^{+} \qquad (13)$$

The corresponding overall equilibrium constant logarithm is given by $\log K^{Cu}_{CuL} - pK^{H}_{CuL} - pK^{H}_{HL}$, all three components of which appear in Tables I and II. For the four dipeptide ligands in the same order as they appear in the tables the overall equilibrium constant logarithm for reaction 13 is −6.59, −7.09,

*This differs from an earlier conclusion [9] for the $Cu^{2+}$/β-alanyl-β-alaninate system, where ionization of the amide group was suggested.

−8.18, and −10.6. These values are within 0.1 log unit of those (−6.65, −7.10, −8.09 and −10.7) calculated from an earlier study [9]. The values provide a quantitative measure of the stabilities of the dipeptide complexes with two linked chelate rings. The order of stabilities is Gly-gly > Gly-β-ala > β-Ala-gly > β-Ala-β-ala. If amino group basicity is omitted as done earlier [9] ($pK^{H}_{HL}$ ignored), then the ordering of the middle two ligands is reversed.

The preceding comparison indicates that the order of decreasing stabilities of dipeptide complexes with two linked chelate rings is 5/5 > 5/6 > 6/5 ≫ 6/6. Each of the two chelate rings contain two trigonal C and N atoms.

In α,ω-diaminocarboxylates the order of decreasing first stability constants is 5/6 > 5/5 > 5/7 for $Co^{2+}$, $Ni^{2+}$, $Cu^{2+}$ and $Zn^{2+}$ [17]. When reference is made to neutral solutions by allowing for amino group basicities the order of decreasing stabilities according to the reaction

$$M^{2+} + H_2L^{+} \rightleftharpoons ML^{+} + 2H^{+}$$

is 5/5 > 5/6 > 5/7 for all four metal ions. This comparison has been made previously for $Co^{2+}$ complexes [18]. In this group of ligands the carbonyl carbon is the only trigonal atom within a chelate ring.

In linear tridentate polyamines with no trigonal atoms the order of decreasing stability constant for the $ML^{2+}$ complex in triamines is 5/6 > 5/5 > 6/6 for $Ni^{2+}$, $Cu^{2+}$, and $Zn^{2+}$ [19], and in tetramines is 5/6/5 > 6/5/6 > 5/5/5 > 6/6/6 for $Ni^{2+}$ and $Cu^{2+}$ (the middle two are reversed for $Zn^{2+}$) [20, 21]. If we refer the uncomplexed ligand to neutral solution and allow for ligand basicity by evaluating the equilibrium constant for the substitution reaction in triamines

$$M^{2+} + H_3L^{3+} \rightleftharpoons ML^{2+} + 3H^{+}$$

and in tetramines

$$M^{2+} + H_4L^{4+} \rightleftharpoons ML^{2+} + 4H^{+}$$

the stability order for all three metal ions $Ni^{2+}$, $Cu^{2+}$, and $Zn^{2+}$ becomes decisively 5/5 > 5/6 > 6/6 in triamines and 5/5/5 > 5/6/5 > 6/5/6 > 6/6/6 in tetramines. This argument has been presented previously for $Cu^{2+}$ complexes [22].

Thus in these systems containing two linked chelate rings each with two trigonal atoms, two linked rings with one trigonal atom in one ring, or two or three linked rings with no trigonal atoms, 5-membered chelate rings are always favored when differing amino group basicities are allowed for in making comparisons.

It is interesting to compare the above conclusion with linked ring systems containing three trigonal carbon and nitrogen atoms. Results reported for a series of tripeptides containing a β-alanine residue [23] may be treated similarly to the dipeptides by considering the overall equilibrium constant:

$$Cu^{2+} + HL \rightleftharpoons Cu(L{-}2H)^{-} + 3H^{+}$$

The tripeptides and their overall equilibrium constant logarithms are: Gly-gly-gly, −14.6; Gly-gly-β-ala, −14.0; Gly-β-ala-gly, −13.6; and β-Ala-gly-gly, −14.9. The values span a narrow range of only 1.3 log units. The order of decreasing stabilities according to ring size of the three linked chelate rings is 5/6/5 > 5/5/6 > 5/5/5 > 6/5/5. The number of trigonal atoms in the three linked rings is 2, 3, 2. Thus when three trigonal atoms appear in a chelate ring of a linked ring complex, 6-membered rings begin to become favored over 5-membered rings.

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