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Solution Equilibria of Histidine-containing Ternary Amino Acid–Copper(II) Complexes in 20 v/v% Dioxane–Water

Osamu Yamauchi,* Toshikazu Takaba, and Takeshi Sakurai†
Faculty of Pharmaceutical Sciences, Osaka University, Yamadakami, Suita, Osaka 565
†*Institute of Chemistry, College of General Education, Osaka University, Toyonaka, Osaka 560*

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Equilibria of histidine-containing ternary amino acid–copper(II) systems have been investigated by potentiometric titration in acid—neutral solution in 20 v/v% dioxane–water containing 0.1 M KNO_3 (1 M=1 mol dm^{-3}) at 25 °C. The stability constants of the binary complexes of histidine (his) and other amino acids (A) with a polar or a nonpolar side chain (A: serine, threonine, homoserine, asparagine, glutamine, citrulline, glycine, alanine, or valine) and the ternary complexes Cu(his)(A) have been calculated by the method of nonlinear least-squares. The overall stability constants for CuA, CuA_2, and Cu(his)(A) are higher than the values reported for the corresponding complexes in water, those for Cu(his) and $Cu(his)_2$ remaining unaffected. The stability constants (log β_{1110}) for Cu(his)(A) are in the range 17.4—18.0, no difference in stability being observed between the diastereomers Cu(L-his)(L-A) and Cu(D-his)(L-A). Species distribution as a function of pH reveals that Cu(his)(A) predominates in the neutral region. The stability constants have been compared with various theoretical values and found to be higher than the statistically estimated ones.

Copper(II) in human blood serum has been reported to form histidine-containing ternary complexes preferentially with threonine (thr), asparagine (asn), and glutamine (gln).[1] In fact a ternary complex, L-histidinato-L-threoninatocopper(II), has been detected by thin-layer chromatography using ^{64}Cu and ^{14}C as tracers.[2] Such low-molecular-weight copper(II) complexes are involved in the copper(II) transport in biological systems.[3,4] Computer simulation of the multimetal-multiligand equilibria in blood plasma indicates that copper(II) exists predominantly as binary and ternary complexes containing histidine (his),[5] supporting the reported involvement of histidine in copper(II) complex formation.

X-Ray crystal structure analysis of [Cu(L-his)(L-thr)(H_2O)]·H_2O shows that histidine coordinates to copper(II) through its amine and imidazole nitrogens with the carboxylate oxygen in the apical position and that threonine occupies the rest of the coordination plane with a glycine-like chelation in a *cis*-configuration with respect to the amino groups.[6]

On the basis of the molecular structure disclosed, the results of synthesis of ternary copper(II) complexes containing histidine and an amino acid with a polar side chain, such as asparagine and serine, and optical resolution of racemic histidine through ternary complex formation, we inferred that such a combination of ligands around copper(II) facilitates the crystallization of the ternary complexes probably owing to ligand-ligand interactions between the carboxylate oxygen of histidine and the polar side group of the other amino acid.[7,8] Very recently the structures of two modifications of L-asparaginato-L-histidinatocopper(II), isolated from water and from aqueous ethanol, have been revealed by X-ray analysis to have different side chain conformations of L-asparagine,[9] showing that the conformation is flexible and affected by the crystalline environment. Since the polar side groups of histidine and asparagine can have access to each other to within a hydrogen bond distance by the conformational change, the structures suggest that ligand-ligand interactions may take place under favorable conditions.

In order to obtain information on the ternary complex formation and ligand-ligand interactions in a medium with low polarity, we have investigated by potentiometric titration in 20 v/v% dioxane–water the equilibria of copper(II) complexes containing histidine and/or other amino acids (A) with a polar or a nonpolar side chain and compared the results with those reported for the systems in water. Comparison was also made between the stability constants for the diastereomers Cu(L-his)(L-A) and Cu(D-his)(L-A) and between the experimental and theoretical values for Cu(L-his)(L-A).

Experimental

Materials. L-Homoserine (L-hmser), L-glutamine, L-citrulline (L-cit), glycine (gly), L-alanine (L-ala), and L-valine (L-val) were obtained from Nakarai Chemicals, Ltd., and L- and D-histidine hydrochloride monohydrate, L-threonine, and L-serine (L-ser) from Ajinomoto Chemical Co. Dioxane (Nakarai Chemicals, Ltd.) was purified by distillation after refluxing for two hours over calcium hydride. All the materials were of analytical reagent grade or of highest grade available. Water was distilled and deionized.

Potentiometric Titrations. *Reagents*: Carbonate-free potassium hydroxide (0.1 M (M=mol dm^{-3})) was prepared by the method of Armstrong[10] and standardized against potassium hydrogen phthalate. Copper (II) nitrate (0.01 M) was standardized by chelatometric titration using EDTA with 1-(2-pyridylazo)-2-naphthol (PAN) as an indicator. Zinc (JIS primary standard) was used for standardization of 0.01 M EDTA.

Apparatus: Measurement of pH was made with an Orion Research 801A digital pH meter equipped with a 90-01-00 glass electrode and a 91-02-00 double junction reference electrode. The meter was standardized with Horiba standard buffer solutions (4.01, 6.86, and 9.18 at 25 °C).

Correction of pH *Meter Reading*: The pH meter readings in 20 v/v% dioxane–water were corrected according to Van Uitert and Haas[11] by titrating HNO_3 in 0.1 M KNO_3 (ionic strength (I)=0.1 M) with 0.1 M KOH. The correction factor C was calculated by means of the relation

$$-\log [H^+] = pH_M + C,$$

where [H^+] and pH_M denote the analytical hydrogen ion concentration and the pH meter reading in 20 v/v% dioxane–

water, respectively. Thus, the hydrogen ion concentration was given by $10^{-\mathrm{pH_M}}/0.66$. The ion product of water ($\mathrm{p}K_\mathrm{w}'$) in this medium ($I=0.1$ M (KNO_3)) was determined to be 14.61 by varying the hydroxide ion concentration with 0.1 M KOH.

Determination of Equilibrium Constants: For determination of the acid dissociation constant ($\mathrm{p}K_\mathrm{a}$), a 4×10^{-3}M solution of an amino acid containing an excessive amount of nitric acid was titrated with 0.1 M KOH at 25±0.05 °C under a nitrogen atmosphere. The data for determination of the stability constants of the binary and ternary complexes were obtained by titrating 1:2 Cu(II)–amino acid systems and 1:1:1 Cu(II)–his–A systems, respectively, under the same conditions, the initial total concentration of copper(II) being 2×10^{-3} M. Dioxane was added at each addition of 0.1 M KOH to maintain the solvent composition. The pH value was measured after each addition of 0.1 M KOH and dioxane. Reproducibility of the titration was checked by multiplicate runs.

Calculations. The equilibria of the present systems in general are expressed by Eq. 1 and the equilibrium constant β_{pqrs} (Eq. 2):

$$p\mathrm{Cu} + q\mathrm{his} + r\mathrm{A} + s\mathrm{H} \overset{\beta_{pqrs}}{\rightleftharpoons} \mathrm{Cu}_p(\mathrm{his})_q(\mathrm{A})_r(\mathrm{H})_s, \qquad (1)$$

$$\beta_{pqrs} = \frac{[\mathrm{Cu}_p(\mathrm{his})_q(\mathrm{A})_r(\mathrm{H})_s]}{[\mathrm{Cu}]^p[\mathrm{his}]^q[\mathrm{A}]^r[\mathrm{H}]^s}, \qquad (2)$$

where p, q, r, and s refer to the numbers of Cu(II), his, A, and H contained in the complex, respectively, and the charges are omitted. The β_{pqrs} values were calculated by the method of nonlinear least-squares by using the computer program SCOGS.[12] The acid dissociation constants were calculated by the usual method and refined by least-squares treatment when necessary.

Results

Acid Dissociation Constants. Typical titration curves for ligands alone are illustrated in Fig. 1, and the acid dissociation constants calculated from the data are summarized in Table 1, where $\mathrm{p}K_\mathrm{COOH}$, $\mathrm{p}K_\mathrm{Im}$, and $\mathrm{p}K_{\mathrm{NH_2}}$, referring to the $\mathrm{p}K_\mathrm{a}$'s of the carboxyl, the imidazole, and the amino group, respectively, are compared with the corresponding values determined in water.[13] The $\mathrm{p}K_\mathrm{COOH}$ values are less reliable owing to the low concentrations of the solutions, the standard deviations being *ca.* ±0.05 log unit. Whereas the constants for the amino groups, $\mathrm{p}K_{\mathrm{NH_2}}$, in aqueous dioxane are higher than those in water by 0.1—0.2 log unit,[13] the imidazole group exhibits a slightly lower $\mathrm{p}K_\mathrm{a}$ value.

Stability Constants. Binary and ternary copper(II)–amino acid systems give the titration curves shown in Fig. 2, which reveals that coordination of the amino acids are nearly complete at neutral pH. The calculated stability constants ($\log\beta_{pqrs}$) for the binary and the ternary complexes are listed in Tables 2 and 3, respectively. In the computation we took into account the complex species, CuA, $\mathrm{CuA_2}$, Cu(his), $\mathrm{Cu(his)_2}$, Cu(his)(H), $\mathrm{Cu(his)_2(H)}$, $\mathrm{Cu(his)_2(H)_2}$, and Cu(his)(A). The stability constants thus determined reproduce the experimental curves satisfactorily (Fig. 3), with the standard deviations expressed in titer in the binary and ternary systems usually less than 0.5 and 1% of the total volume of 0.1 M KOH used, respectively. We see from Table 2 that the binary complexes, CuA and $\mathrm{CuA_2}$, and the protonated complexes of histidine exhibit higher stability constants in aqueous dioxane than in water,[13,14] while Cu(his) and $\mathrm{Cu(his)_2}$ remain equally stable in both media. The ternary complexes Cu(his)(A) have the $\log\beta_{1110}$ values of 17.4—18.0 (Table 3), which are also higher than the corresponding values in water.[8,14] The diastereomers Cu(L-his)(L-A) and Cu(D-his)(L-A) have $\log\beta_{1110}$ values which agree with each other to within experimental errors, apparently showing no stereo-

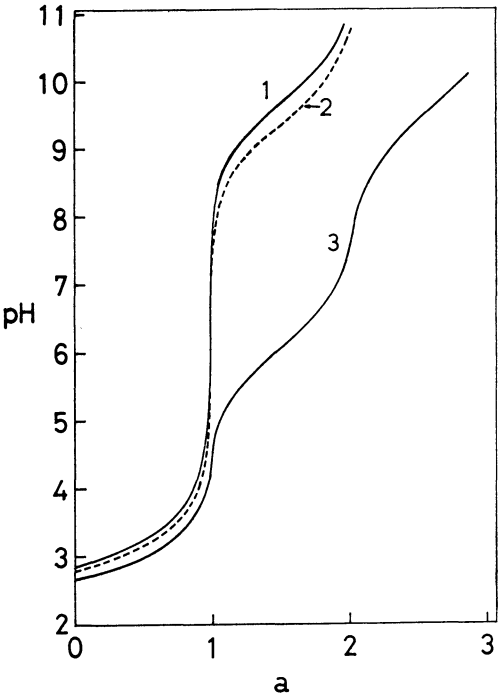


Fig. 1. Typical titration curves for ligands (25 °C; $I=0.1$M (KNO_3)). Curve 1, L-cit; curve 2, L-ser; curve 3, L-his. Initial total concentration: 0.004000 M. a: Moles of KOH added per mole of amino acid.

Table 1. Acid dissociation constants of amino acids in 20 v/v% dioxane–water (25 °C; $I=0.1$ M (KNO_3))[a)]

Amino acid	Acid dissociation constant[b)]		
	$\mathrm{p}K_\mathrm{COOH}$	$\mathrm{p}K_\mathrm{Im}$	$\mathrm{p}K_{\mathrm{NH_2}}$
gly	2.6_7(2.36)		9.74(9.57)
L-ala	2.6_4(2.30)		9.82(9.69)
L-val	2.6_2(2.26)		9.68(9.49)
L-asn	2.4_6(2.14)		8.89(8.72)
L-gln	2.4_5(2.17)		9.14(9.01)
L-cit	2.5_8(2.43)		9.49(9.41)
L-ser	2.4_4(2.13)		9.16(9.06)
L-thr	2.5_0(2.21)		9.07(8.97)
L-hmser	2.4_6(2.27)		9.44(9.28)
L-his	1.8 (1.70)	5.93(6.02)[c)]	9.23(9.08)[c)]

a) Standard deviations are <0.05 for $\mathrm{p}K_\mathrm{COOH}$ and <0.01 for $\mathrm{p}K_\mathrm{Im}$ and $\mathrm{p}K_{\mathrm{NH_2}}$. b) Corresponding values in water taken from Ref. 13 are shown in parentheses. c) We obtained the following values in water: $\mathrm{p}K_\mathrm{Im}=6.03$; $\mathrm{p}K_{\mathrm{NH_2}}=9.11$(25 °C; $I=0.1$M (KNO_3)).

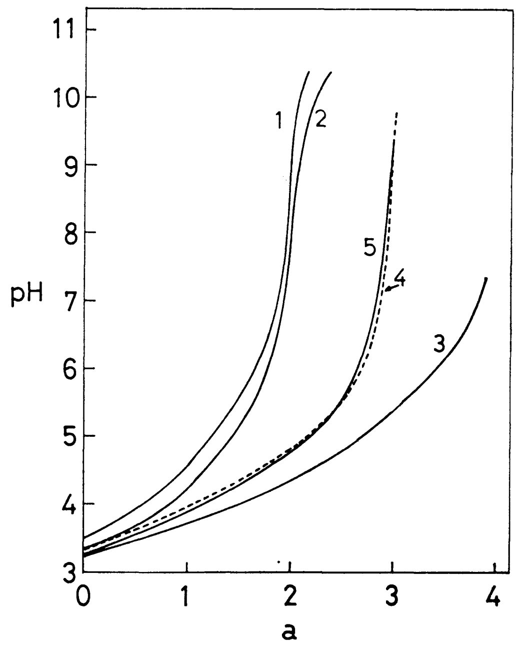


Fig. 2. Typical titration curves for binary and ternary copper(II)–amino acid systems (25°C; I=0.1 M (KNO$_3$)). Curves 1—5 correspond to the following systems: 1, 1:2 Cu(II)–L-hmser; 2, 1:2 Cu(II)–L-asn; 3, 1:2 Cu(II)–L-his; 4, 1:1:1 Cu(II)–L-his–L-hmser; 5, 1:1:1 Cu(II)–L-his–L-asn.

Initial total concentrations are as follows:

(i) Binary systems Cu(II)=0.002016 M; his or A=0.004000 M.

(ii) Ternary systems Cu(II)=0.002016 M; his=0.002000 M; A=0.002000 M.

a: Moles of KOH added per mole of Cu(II).

Table 2. Stability constants of binary copper(II) complexes in 20 v/v% dioxane-water (25°C; I=0.1 M(KNO$_3$))[a)]

Amino acid	Stability constant[b)]	
	$\log \beta_{1010}$	$\log \beta_{1020}$
gly	8.55(8.15)	15.64(15.03)
L-ala	8.40(8.13)	15.45(14.92)
L-val	8.39(8.11)	15.52(14.90)
L-asn	8.20(7.86)	14.95(14.42)
L-gln	8.02(7.75)	14.80(14.23)
L-cit	8.29(7.92)	15.19(14.39)
L-ser	8.14(7.89)	14.98(14.48)
L-thr	8.28(8.01)	15.21(14.73)
L-hmser	8.32(7.97)	15.24(14.69)
L-his[c)]	10.13(10.10)	18.13(18.10)

a) Standard deviations are <0.01 for CuA and CuA$_2$ and 0.02—0.04 for the histidine complexes. b) Corresponding values in water taken from Ref. 13 are shown in parentheses. The values for the histidine complexes were taken from Ref. 14. c) $\log \beta_{1100}$ and $\log \beta_{1200}$ values. The stability constants for the protonated complexes are as follows: $\log \beta_{1101}$=14.31 (14.00); $\log \beta_{1201}$=24.07 (23.88); $\log \beta_{1202}$=27.83 (27.56).

Table 3. Stability constants of ternary complexes Cu$_p$(his)$_q$(A)$_r$(H)$_s$ in 20 v/v% dioxane-water (25 °C; I=0.1 M (KNO$_3$))[a)]

Amino acid	Stability constant ($\log \beta_{1110}$)	
	Cu(L-his)(A)	Cu(D-his)(A)
gly	17.78(0.01)	
L-ala	17.83(0.01)	17.80(0.01)
L-val	18.01(0.01)	18.04(0.02)
L-asn	17.53(0.03)	17.54(0.03)
L-gln	17.42(0.01)	17.45(0.01)
L-cit	17.67(0.02)	17.64(0.02)
L-ser	17.51(0.02)	17.54(0.02)
L-thr	17.57(0.02)	17.59(0.02)
L-hmser	17.64(0.01)	17.68(0.01)

a) Standard deviations are shown in parentheses.

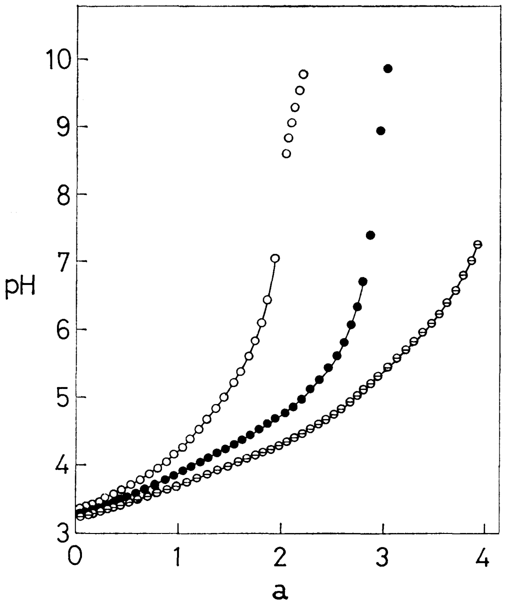


Fig. 3. Fit of the theoretical titration curves to the experimental.

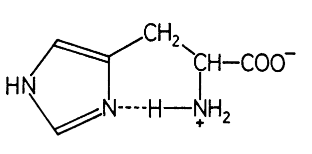
Experimental: ○ 1:2 Cu(II)–L-asn, ⊖ 1:2 Cu(II)–L-his, ● 1:1:1 Cu(II)–L-his–L-asn.

Theoretical ——.

selectivity in complex formation. In this connection, Freeman and Martin[15)] and Brookes and Pettit[14)] also reported that no stereoselectivity is observed between the diastereomers of Cu(his)(thr) in water, but the latter observed a small stability difference (0.06 log unit) between Cu(L-his)(L-val) and Cu(D-his)(L-val).

Discussion

Equilibrium Constants in 20 v/v% Dioxane–Water. Binary Systems: Depending on the structure of molecules involved, the polarity of the medium affects their behavior in different ways. The use of aqueous dioxane in place of water could approximate biological fluids, where the polarity may be lower because of proteins and other dissolved molecules and the difference in the stability of complexes could be more pronounced. The slightly lower pK_a value of the imidazole group of histidine as compared with the value in water may be explained by the result of a recent NMR study of histidine in aqueous solution,[16)] which indicates that neutral histidine favors the struc-



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ture with an intramolecular hydrogen bonding (**1**). Deprotonation from the imidazolium group of monoprotonated histidine is probably more favorable in aqueous dioxane and in hydrophobic regions in biological systems where the hydrogen bonding in **1** is more stabilized than in water. Analogously the higher log β_{1010} and log β_{1020} values of the complexes CuA and CuA_2 are interpreted as due to stabilization of the glycine-like mode of coordination involving a metal-carboxylate bonding with ionic character.

On the other hand, the copper(II)-histidine complexes behave differently; Cu(his) and $Cu(his)_2$ have approximately the same stability constants as those in water. This is what we expect for the two nitrogen-copper(II) bonds with covalent character through the amino group and the imidazole moiety. Formation of Cu(his)(H) and $Cu(his)_2(H)$ from Cu(his) and $Cu(his)_2$, respectively, is accompanied by stability enhancement (*ca.* 0.3 log unit) due to the solvent effect. This suggests that the carboxylate oxygen of histidine is involved in the coordination plane of copper(II).[17–20]

Ternary Systems: For the ternary complexes to have an intramolecular ligand-ligand interaction, the active complex Cu(L-his)(L-A) should assume a *cis*-configuration and the *meso* complex Cu(D-his)(L-A) a *trans*-configuration with respect to the amino groups, because of the steric requirement for the interaction. Lack of stereoselectivity may implicate either that the intramolecular interaction is too weak to affect the stability and/or *cis-trans* isomerism to an appreciable extent under the present conditions or that the proposed *cis*- and *trans*-isomers have the same stability. For the ternary systems with an acidic amino acid (Ac) and a basic amino acid (B) such as Cu(lys)(glu)(H), where lys and glu refer to lysine and glutamic acid, respectively, the difference log β_{1111}—log β_{1110} is larger than that for the system involving alanine instead of Ac.[21] This has been interpreted as due to the intramolecular electrostatic interaction between the oppositely charged groups of the side chains of Ac and B, because it makes the deprotonation from the ω-ammonium group of B more unfavorable than when Ac is replaced with alanine. However, no stereoselectivity has been detected between the diastereomers of Cu(Ac)(B)(H). Considering that the intramolecular hydrogen bonding proposed for Cu(his)(A) is weaker than the electrostatic interactions in Cu(Ac)(B)(H), it may not affect the stability constants appreciably even in 20 v/v% dioxane-water.

Information is lacking about the structures in solution of the ternary complexes, especially the *meso* complexes; they might exist as mixtures of geometric isomers. As regards the active complexes, however, the X-ray crystal structure analyses,[6,9] which have disclosed that all the three histidine-containing ternary copper(II) complexes have a *cis*-arrangement of the amino groups, may indicate their plausible structures in solution.[8]

Preferential Formation of Ternary Complexes. The species distributions as a function of pH, illustrated for Cu(L-his)(L-ala), Cu(L-his)(L-hmser), and Cu(L-his)(L-cit) in Fig. 4, show that the ternary complex Cu(his)(A) predominates in the neutral region.

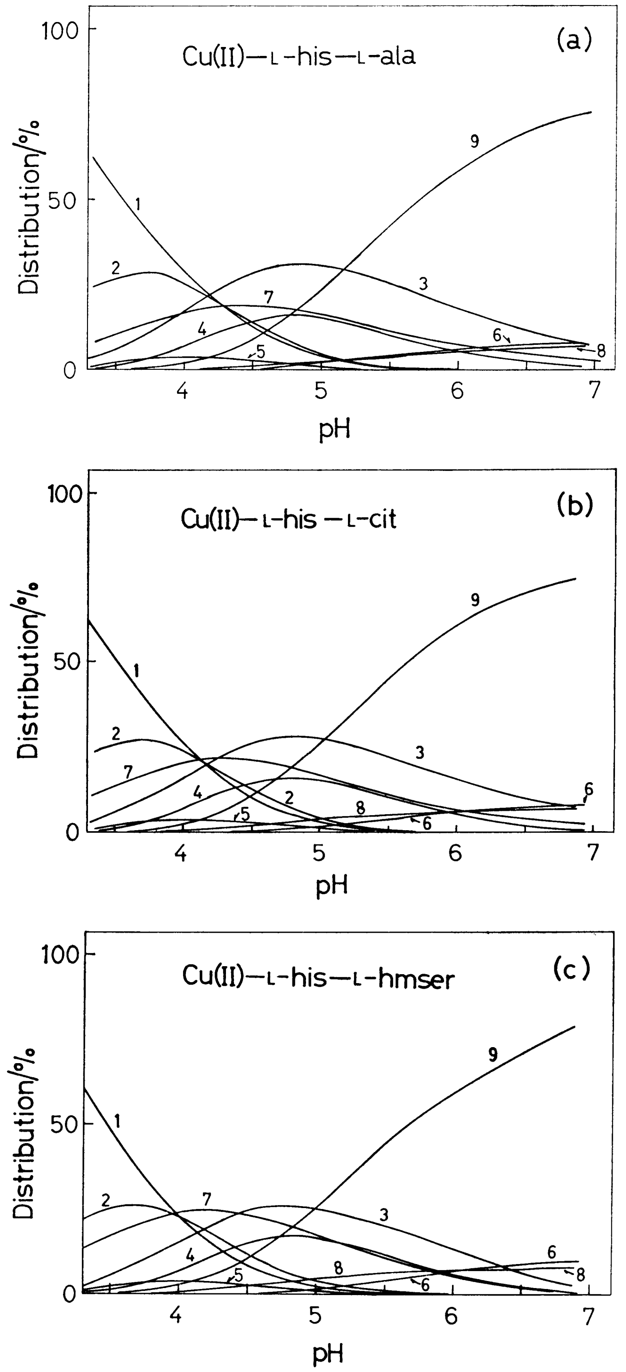

Fig. 4. Species distributions in the ternary systems as a function of pH(25 °C; I=0.1 M (KNO_3)).
(a) 1:1:1 Cu(II)-L-his-L-ala; (b) 1:1:1 Cu(II)-L-his-L-cit; (c) 1:1:1 Cu(II)-L-his-L-hmser.
Curves 1—9 correspond to the following species: 1, Cu(II); 2, Cu(L-his)(H); 3, Cu(L-his); 4, $Cu(L\text{-}his)_2$-(H); 5, $Cu(L\text{-}his)_2$-$(H)_2$; 6, $Cu(L\text{-}his)_2$; 7, Cu(L-A); 8, $Cu(L\text{-}A)_2$; 9, Cu(L-his)-(L-A).
Initial total concentrations: Cu(II)=0.002016 M; L-his=0.002000 M; L-A=0.002000 M.

Whether the formation of a ternary complex is favored or not as compared with that of binary complexes can be estimated in various ways. We use here $\log X$ and $\log \beta_{\mathrm{stat}}$ defined by Eqs. 4 and 5, respectively:

$$\mathrm{Cu(his)_2} + \mathrm{CuA_2} \overset{X}{\rightleftharpoons} 2\mathrm{Cu(his)(A)}, \tag{3}$$

$$\log X = 2\log\beta_{1110} - (\log\beta_{1200} + \log\beta_{1020}), \tag{4}$$

$$\log\beta_{\mathrm{stat}} = 1/2(\log\beta_{1200} + \log\beta_{1020}) + \log 2, \tag{5}$$

$$\Delta\log\beta = \log\beta_{1110} - \log\beta_{\mathrm{stat}}. \tag{6}$$

We prefer the use of $\log X$ to $\Delta\log K$ ($=\log\beta_{1110} - (\log\beta_{1100} + \log\beta_{1010})$), because electrostatic factors cancel in the $\log X$ formulation for the present amino acid–copper(II) complexes where the charges of the two ligands are equal to each other.[22] The situation is regarded as the same in the $\log\beta_{\mathrm{stat}}$ formulation. The observed values of $\log X$ and $\log\beta_{1110}$ can be compared with the statistical values of 0.6[23,24] and $\log\beta_{\mathrm{stat}}$,[25] respectively. For all the systems investigated the values are larger than the statistical values ($\log X > 0.6$ and $\Delta\log\beta > 0$) (Table 4). Thus, the formation of the ternary complexes with histidine is favored irrespective of the group contained in the side chain of A. This is partly ascribable to the favorable combination of the π-system of imidazole with a ligand with an oxygen donor.[26]

An interesting approach to the estimation of stability constants has been made by Tanaka,[27,28] who evaluated them on the basis of mechanistic considerations and by introduction of several parameters describing possible ligand-ligand interactions through the central metal ion. For copper(II) complexes with at least one neutral amine nitrogen as a ligand, the following relationship is derived:[28]

$$\mathrm{Cu(his)} + \mathrm{A} \overset{K}{\rightleftharpoons} \mathrm{Cu(his)(A)}, \tag{7}$$

$$\log K_{\mathrm{calcd}} = \log\beta_{1010} + \sum^{\mathrm{I}}\sum^{\mathrm{J}} \delta_{\mathrm{ij}}\mathrm{X_i(his)Y_j(A)}, \tag{8}$$

where δ_{ij} is the ligand interaction term allowing for the effect of the donor atom $\mathrm{X_i}$ of his on the donor atom $\mathrm{Y_j}$ of A, and $\mathrm{X_i(his)}$ and $\mathrm{Y_j(A)}$ are the numbers of donor atoms $\mathrm{X_i}$ and $\mathrm{Y_j}$, respectively. Since $\log K = \log\beta_{1110} - \log\beta_{1100}$, we have from Eq. 8

$$\log\beta_{1110\,\mathrm{calcd}} = \log\beta_{1100} + \log\beta_{1010} + \sum^{\mathrm{I}}\sum^{\mathrm{J}} \delta_{\mathrm{ij}}\mathrm{X_i(his)Y_j(A)}. \tag{9}$$

The δ_{ij} values have been determined by a detailed survey of reported stability constants.[28] For Cu(his)(A) with an aromatic nitrogen ($\mathrm{N_{Im}}$) and an aliphatic nitrogen ($\mathrm{N_{Am}}$) of histidine and an oxygen and an aliphatic nitrogen of A in the coordination plane, the expected values $\log\beta_{1110\,\mathrm{calcd}}$ can be calculated by using $\delta_{\mathrm{N_{Im}N_{Am}}} = -0.25$, $\delta_{\mathrm{N_{Im}N_{Im}}} = -0.39$, $\delta_{\mathrm{N_{Im}O}} = 0.09$, $\delta_{\mathrm{N_{Am}N_{Am}}} = -0.35$, and $\delta_{\mathrm{N_{Am}O}} = -0.26$. Although these δ_{ij} values refer to the systems in water, we have calculated the $\log\beta_{1110\,\mathrm{calcd}}$ values according to Eq. 9 by assuming that the δ_{ij} values are the same in 20 v/v% dioxane–water as in water because they are essentially the differences between the calculated and experimental stability constants. We see from Table 4 that the estimated values for Cu(his)(A) are in excellent agreement with the experimental ones, indicating that the δ_{ij} values hold in 20 v/v% dioxane–water and that the stability of these complexes could be affected by the electronic ligand-ligand interactions mediated by copper(II).

For the binary complexes, $\mathrm{CuA_2}$, the values calculated by Eq. 10[28] are also close to those determined experimentally:

$$\log\beta_{1020\,\mathrm{calcd}} = 2\log\beta_{1010} + \sum^{\mathrm{I}}\sum^{\mathrm{J}} \delta_{\mathrm{ij}}\mathrm{X_i(A)Y_j(A)} - \log 2. \tag{10}$$

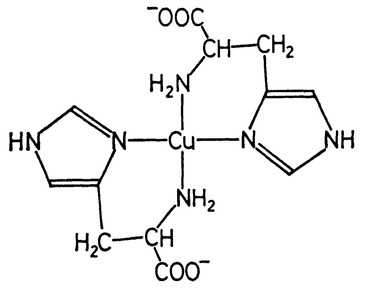

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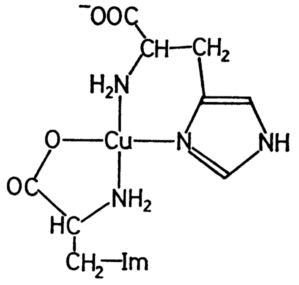
TABLE 4. COMPARISON OF STABILITY CONSTANTS WITH THEORETICAL VALUES

Amino acid	Cu(L-his)(L-A)					Cu(L-amino acid)$_2$	
	$\log\beta_{1110}$	$\log\beta_{1110\,\mathrm{calcd}}$[a]	$\log\beta_{\mathrm{stat}}$[b]	$\Delta\log\beta$[c]	$\log X$[d]	$\log\beta_{1020}$	$\log\beta_{1020\,\mathrm{calcd}}$
gly	17.78	17.91	17.18	0.60	1.79	15.64	15.64
ala	17.83	17.76	17.09	0.74	2.08	15.45	15.34
val	18.01	17.75	17.13	0.88	2.37	15.52	15.32
asn	17.53	17.56	16.84	0.69	1.98	14.95	14.94
gln	17.42	17.38	16.77	0.65	1.91	14.80	14.58
cit	17.67	17.65	16.96	0.71	2.02	15.19	15.12
ser	17.51	17.50	16.86	0.65	1.91	14.98	14.82
thr	17.57	17.64	16.97	0.60	1.80	15.21	15.10
hmser	17.64	17.68	16.99	0.65	1.91	15.24	15.18
his[f]						18.13	18.72[g] 19.19[h]

a) Calculated according to Eq. 9. b) Calculated according to Eq. 5. c) Calculated according to Eq. 6. d) Calculated according to Eq. 4. e) Calculated according to Eq. 10. f) $\log\beta_{1200}$ and $\log\beta_{1200\,\mathrm{calcd}}$ values. g) Two ($\mathrm{N_{Im}}$)($\mathrm{N_{Am}}$) coordinations assumed. h) One ($\mathrm{N_{Im}}$)($\mathrm{N_{Am}}$) and one ($\mathrm{N_{Am}}$)(O) coordination assumed.



3

Interestingly Cu(his)$_2$ has a log β_{1200} value much smaller than the values estimated from log β_{1100} by Eq. 10 for two sets of donor atoms in the coordination plane, *i.e.*, two $(N_{Im})(N_{Am})$ coordinations (**2**) and one $(N_{Im})(N_{Am})$ and one (N_{Am})(O) coordination (**3**). This suggests that the sterically and/or electronically unfavorable combination of donor groups in Cu(his)$_2$ contributes to the formation of ternary complexes.

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