# Solution Equilibria of Ternary α-Amino Acid–Copper(II) Complexes with Electrostatic Ligand-Ligand Interactions

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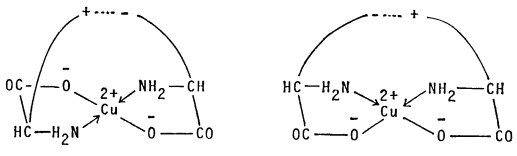
(Received February 16, 1978)

The stability constants for the parent and mixed copper(II) complexes involving an acidic α-amino acid (aspartic acid or glutamic acid, abbreviated as A) and/or a basic α-amino acid (arginine, lysine, or ornithine, abbreviated as B) have been determined by potentiometric titration at 25 °C in 0.1 and 0.03 M $KNO_3$ (M=mol dm$^{-3}$). The protonated ternary species Cu(L-A)(L-B)(H) and Cu(D-A)(L-B)(H) (species 1111) have essentially the same stability constants, the log $\beta_{1111}$ values being in the range 25.0—27.5. The species distribution curves for the systems Cu(II)–L- or D-glutamic acid–L-lysine at the ionic strength $I$=0.1 and 0.03 reveal that species 1111 is more predominant at $I$=0.03 than at $I$=0.1, suggesting that electrostatic ligand-ligand interactions between the carboxylate group in the side chain of A and the ammonium or the guanidinium group in the side chain of B are reinforced in solution at lower ionic strength to favor the ternary complex formation. The constants for deprotonation from species 1111 to form 1110, *e.g.* Cu(L-A)(L-B), are given by the difference log $\beta_{1111}$ − log $\beta_{1110}$, which is higher for the Cu(II)–A–B systems than for the systems containing alanine in place of A. The results indicate that intramolecular electrostatic interactions are present in the ternary species 1111, stabilizing them under favorable conditions.

Ternary metal complexes have been extensively studied because of their possible significance as models for biological processes assisted by metal ions. Since non-covalent interactions are supposed to be an important source of specificity or selectivity in biological reactions,[1] mixed ligand copper(II) complexes involving aromatic ring stacking[2] and electrostatic ligand-ligand interactions[3] have attracted considerable attention.

On the basis of the electrostatic enzyme-substrate interactions found in the carboxypeptidase A–glycyl-L-tyrosinate complex, we designed mixed amino acid–copper(II) complex systems capable of forming intramolecular electrostatic bonds between the two amino acids, and inferred from synthetic and spectroscopic studies that such ligand-ligand interactions exist in the systems containing an acidic and a basic amino acid.[3] The IR spectra of the isolated complexes[3b] and successful optical resolution of acidic and basic amino acids *via* complex formation[5] indicate that the diastereomers Cu(L-A)(L-B)(H) and Cu(D-A)(L-B)(H), where A refers to aspartate (asp) or glutamate (glu), B to argininate (arg), lysinate (lys), or ornithinate (orn), and H to proton, are probably *cis-trans* isomers arising from the steric requirements for the ligand-ligand interactions (Scheme 1).



Cu(L-A)(L-B)(H) Cu(D-A)(L-B)(H)

Scheme 1.

Brookes and Pettit[6] determined the stability constants for the ternary systems containing copper(II), L- or D-histidine (L- or D-his), and a basic amino acid (L-B), and found the stability differences between the complexes Cu(L-his)(L-B)(H) and Cu(D-his)(L-B)(H), which they ascribed to the intramolecular electrostatic bonding. Steric hindrance has been reported to give rise to stereoselectivity in the ternary copper(II) complexes of *N*-substituted α-amino acids with α-amino acids.[7] Although many other mixed amino acid–copper(II) systems have been studied potentiometrically,[8] no systematic investigations of the ternary systems, copper(II)–L-A–L-B, seem to have been reported. In order to clarify the effects of electrostatic ligand-ligand interactions on the stability of the metal complexes in solution and the factors affecting the optical resolution of DL-A and DL-B, we have carried out a potentiometric study of the ternary amino acid–copper(II) complexes containing A and B.

## Experimental

*Materials.* L-Arginine hydrochloride, L-lysine hydrochloride, L-ornithine hydrochloride, L- and D-aspartic acid, and L- and D-glutamic acid of grade A from Nakarai Chemicals, Ltd. were dried *in vacuo* over $P_4O_{10}$ before use. All other chemicals used were of highest grade available. Water was distilled and deionized.

pH *Titrations.* *Reagents*: Carbonate-free potassium hydroxide (0.1 M, M=mol dm$^{-3}$) was prepared by the method of Armstrong,[9] standardized against potassium hydrogen phthalate, and stored under a nitrogen atmosphere. Copper(II) nitrate (0.01 M) was prepared by dissolving copper(II) nitrate trihydrate in water and standardized against standard zinc by chelatometric titration.

*Apparatus*: An Orion Research 801A digital pH meter equipped with a 90-01-00 glass electrode and a 91-02-00 double junction reference electrode was used for measuring the pH values after standardization with Horiba standard buffer solutions (pH 4.01, 6.86, and 9.18).

*Procedure*: For determination of the acid dissociation constants of the ligands, an aqueous solution ($4\times10^{-3}$ M) of protonated A or B was titrated with 0.1 M KOH at 25± 0.05 °C under a nitrogen atmosphere. For determination of the stability constants of the binary and the ternary systems, 1:2 copper(II)–A or B and 1:1:1 copper(II)–A–B solutions, respectively, were titrated with 0.1 M KOH at a constant copper(II) concentration of $2\times10^{-3}$M under the same conditions. Titration was carried out in the presence of 0.1 or 0.03 M $KNO_3$. Aliquots of A and B to be titrated were taken from stock solutions freshly prepared before use. The

pH value was measured for each addition of a small amount of 0.1 M KOH. The apparent ion products of water, $pK_w'$, at the ionic strength $I=0.1$ and 0.03 were determined to be 13.90 and 14.02, respectively, by titration of 0.01 M $HNO_3$ with 0.1 M KOH, which also gave the hydrogen ion concentrations, $[H^+]$, as $10^{-pH}/0.865$ at $I=0.1$ and $10^{-pH}/0.902$ at $I=0.03$.

## Results and Discussion

*Calculations.* The equilibria of binary and ternary systems and the stability constants $\beta_{pqrs}$ are expressed by

$$pCu + qA + rB + sH \overset{\beta_{pqrs}}{\rightleftharpoons} Cu_pA_qB_rH_s, \quad (1)$$

$$\beta_{pqrs} = \frac{[Cu_pA_qB_rH_s]}{[Cu]^p[A]^q[B]^r[H]^s},$$

where $p$, $q$, $r$, and $s$ denote the numbers of copper(II), A, B, and H, respectively, contained in the complex (charges are omitted). Each species will be represented as *pqrs* hereinafter.

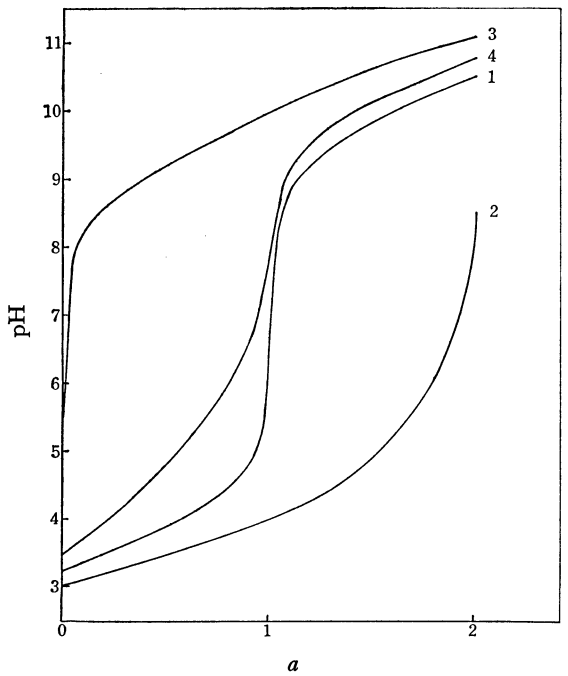



Fig. 1. Typical titration curves for ligands and 1:2 binary systems, Cu(II)-A or B(25 °C; $I=0.1$($KNO_3$)). Total concentrations: ligand=0.004000 M; Cu(II)=0.002016 M.
Curves 1—4 correspond to the following systems: 1, L-asp; 2, Cu(II)-L-asp; 3, L-lys; 4, Cu(II)-L-lys.
*a*: Moles of alkali added per mole of ligand.

The stability constants were calculated from the pH titration data by non-linear least-squares refinement with the use of the computer program SCOGS.[10] The initial estimates for the refinements were taken from the literature[11] or guessed from the values for analogous systems. At the final stage of refinement the difference between experimental and calculated titers were well within 0.02 ml in the pH ranges considered. The acid dissociation constants ($pK_a$) of the ligands were calculated separately with the aid of a computer.

*Stability Constants of Binary Copper(II) Complexes.* Typical titration curves for the systems with a ligand alone and with copper(II) and a ligand are shown in Fig. 1. Table 1 summarizes the $pK_a$ values of the amino acids, most of which are in good agreement with the reported values.[6b,11] To avoid erroneous results suspected at low concentrations of solutions,[9] we did not determine the constants for the α-carboxyl groups of all the amino acids whose acid dissociation occurs at around pH 2. For calculations of the stability constants, such $pK_a$ values were taken from the litera-

Table 1. Acid dissociation constants of ligands (25 °C; $I=0.1$($KNO_3$))[a]

| Ligand | $pK_{a_1}$ | $pK_{a_2}$ |
|---|---|---|
| L-asp | 9.75 | 3.79 |
| D-asp | 9.79 | 3.77 |
| L-glu | 9.61 | 4.15 |
| D-glu | 9.68 | 4.21 |
| L-arg | 12.11 | 9.07 |
| L-lys | 10.85 | 9.14 |
| L-orn | 10.52[b] | 8.75[b] |

a) Standard deviations of all the constants are within ±0.01. b) Taken from Ref. 6b.

Table 2. Stability constants for binary copper(II) complexes containing acidic amino acids (25 °C; $I=0.1$ ($KNO_3$))[a]

| Ligand | Stability | | |
|---|---|---|---|
| | $\log\beta_{1101}$ | $\log\beta_{1100}$ | $\log\beta_{1200}$ |
| L-asp | 12.72(0.009) | 9.00(0.004) | 15.84(0.010) |
| D-asp | 12.70(0.009) | 8.97(0.004) | 15.81(0.009) |
| L-glu | 12.50(0.003) | 8.30(0.001) | 14.80(0.003) |
| D-glu | 12.49(0.005) | 8.32(0.003) | 14.86(0.005) |

a) Standard deviations are shown in parentheses.

Table 3. Stability constants for binary copper(II) complexes containing basic amino acids (25 °C; $I=0.1$ ($KNO_3$))[a]

| Ligand | Stability | | | | $pK_{c_1}$ | $pK_{c_2}$ |
|---|---|---|---|---|---|---|
| | $\log\beta_{1011}$ | $\log\beta_{1022}$ | $\log\beta_{1021}$ | $\log\beta_{1020}$ | | |
| L-arg | 19.55(0.06) | 37.91(0.08) | — | — | — | — |
| L-lys | 18.46(0.007) | 35.63(0.008) | 25.64(0.010) | 15.07(0.008) | 9.99 | 10.57 |
| L-orn | 17.95(0.006) | 34.65(0.007) | 25.73(0.008) | 15.71(0.008) | 8.92 | 10.02 |

a) Standard deviations are shown in parentheses.

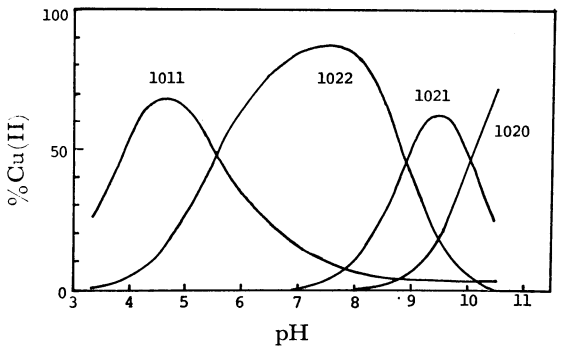



Fig. 2. Species distribution curves for the 1 : 2 Cu(II)-L-orn system (25 °C; $I$=0.1($KNO_3$)).
Total concentrations: L-orn=0.004000 M; Cu(II)=0.002016 M.

ture[11] when necessary. However, they did not affect the present calculations seriously.

The stability constants for the binary complexes are shown in Tables 2 and 3. The titration curves for the binary copper(II)–A systems were satisfactorily reproduced by considering species 1101, 1100, and 1200 excluding the protonated bis-complexes 1202 and 1201. Although Clarke and Martell[12] detected species 1021 in the Cu(II)–arg system at pH ≈11, the present results are explained by the protonated species 1011 and 1022, deprotonation from which was not observed because of precipitation around this pH. On the other hand, the deprotonated species were found to be formed appreciably in the systems Cu(II)–orn and Cu(II)–lys at pH>8, which is illustrated in the species distribution for the 1 : 2 Cu(II)–L-orn system plotted against pH (Fig. 2). The standard deviations expressed in titer were usually larger for the systems with arg than for the systems with the other ligands probably owing to the errors in the pH measurement in a strongly alkaline solution.

Deprotonations from species 1022, where the proton is bound to the ω-amino group, to give 1021 and 1020 are represented by

$$Cu(L\text{-}B)_2(H)_2 \overset{K_{c1}}{\rightleftharpoons} Cu(L\text{-}B)_2(H) + H, \quad (2)$$

$$Cu(L\text{-}B)_2(H) \overset{K_{c2}}{\rightleftharpoons} Cu(L\text{-}B)_2 + H. \quad (3)$$

The dissociation constants $pK_{c_1}$ (=$-\log K_{c_1}$) and $pK_{c_2}$ are given by the differences $\log\beta_{1022}-\log\beta_{1021}$ and $\log\beta_{1021}-\log\beta_{1020}$, respectively. The $pK_{c_1}$ values are lower than the $pK_{a_1}$ values of orn and lys (Tables 1 and 3), and the Cu(II)–orn system has a smaller $pK_{c_1}$ value than that of the Cu(II)–lys system, indicating that orn coordinates to copper(II) at the apical position through its δ-amino group, whereas lys does not coordinate in the same way. This is in line with the ORD[13] and CD[3a,14] spectral behaviors of these systems. Because the $pK_{c_2}$ values for both systems do not differ very much from the respective $pK_{a_1}$ values, they suggest that the δ-amino group of the second orn is not effectively involved in the apical coordination.

*Stability Constants of Ternary Copper(II) Complexes.* The stability constants for the systems were obtained from the titration curves such as those shown in Fig. 3 by the least-squares refinement starting from known

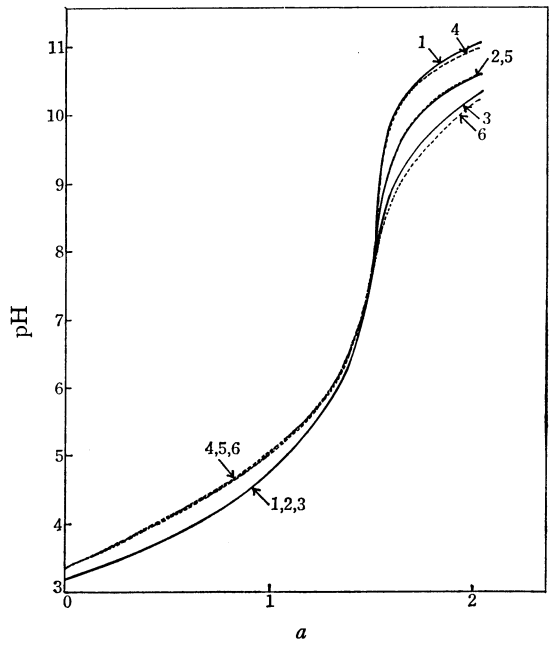



Fig. 3. Titration curves for the 1 : 1 : 1 ternary systems, Cu(II)-L-A-L-B(25 °C; $I$=0.1($KNO_3$)).
Total concentrations: each ligand=0.002000 M; Cu(II)=0.002016 M.
Curves 1—6 correspond to the following systems: 1, Cu(II)-L-asp-L-arg; 2, Cu(II)-L-asp-L-lys; 3, Cu(II)-L-asp-L-orn; 4, Cu(II)-L-glu-L-arg; 5, Cu(II)-L-glu-L-lys; 6, Cu(II)-L-glu-L-orn.
$a$: Moles of alkali added per mole of ligand.

Table 4. Stability constants for ternary copper(II) complexes (25 °C; $I$=0.1 ($KNO_3$))[a]

| Ligand | | Stability | |
|---|---|---|---|
| B | A | $\log\beta_{1111}$ | $\log\beta_{1110}$ |
| L-arg | L-asp | 27.43(0.08) | — |
| L-arg | D-asp | 27.47(0.02) | — |
| L-arg | L-glu | 26.61(0.13) | — |
| L-arg | D-glu | 26.61(0.08) | — |
| L-lys | L-asp | 26.32(0.01) | 15.82(0.02) |
| L-lys | D-asp | 26.27(0.03) | 15.67(0.06) |
| L-lys | L-glu | 25.60(0.01) | 15.08(0.02) |
| L-lys | D-glu | 25.60(0.01) | 15.14(0.02) |
| L-lys | L-ala | 25.52(0.04) | 15.59(0.03) |
| L-orn | L-asp | 25.65(0.04) | 15.29(0.13) |
| L-orn | D-asp | 25.66(0.04) | 15.28(0.17) |
| L-orn | L-glu | 24.93(0.05) | 14.68(0.26) |
| L-orn | D-glu | 24.88(0.06) | — |
| L-orn | L-ala | 24.86(0.07) | 15.37(0.09) |
| L-lys | L-glu | 25.92(0.02)[b] | 15.85(0.03)[b] |
| L-lys | D-glu | 25.98(0.02)[b] | 15.95(0.03)[b] |

a) Standard deviations are shown in parentheses. b) Determined at $I$=0.03($KNO_3$).

stability constants for protonated ligands and binary complexes and estimated constants for ternary complexes. The refined values are given in Table 4 together with those of the systems involving L-alanine (L-ala) in place of L-A.[15]

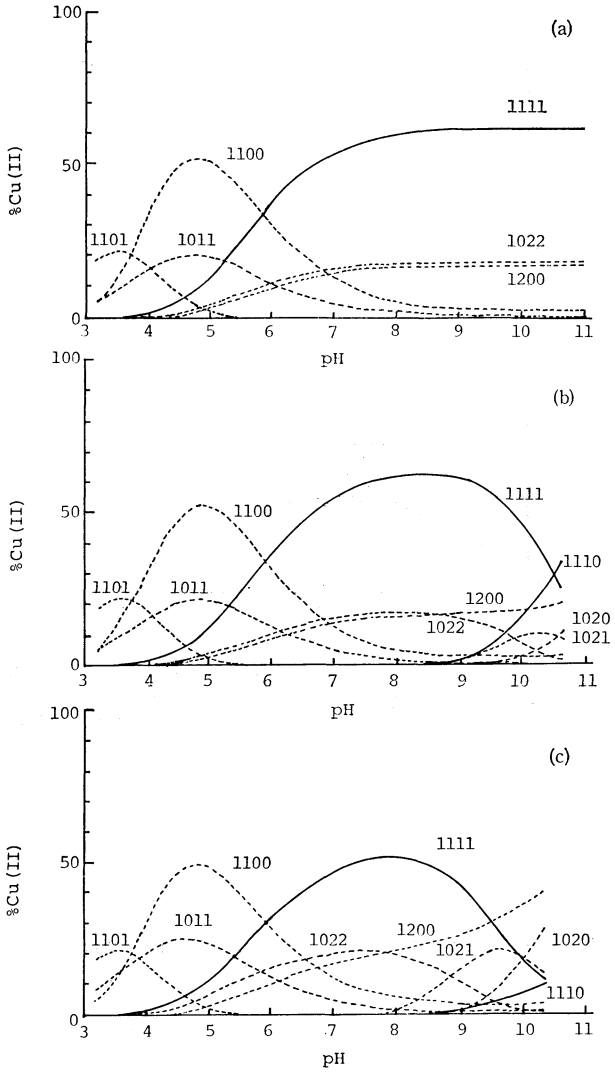



Fig. 4. Species distribution curves for the 1:1:1 Cu(II)–L-asp–L-B systems (25 °C; $I$=0.1 ($KNO_3$)).
Total concentrations: each ligand=0.002000 M; Cu(II)=0.002016 M.
(a): Cu(II)–L-asp–L-arg, (b): Cu(II)–L-asp–L-lys, (c): Cu(II)–L-asp–L-orn.

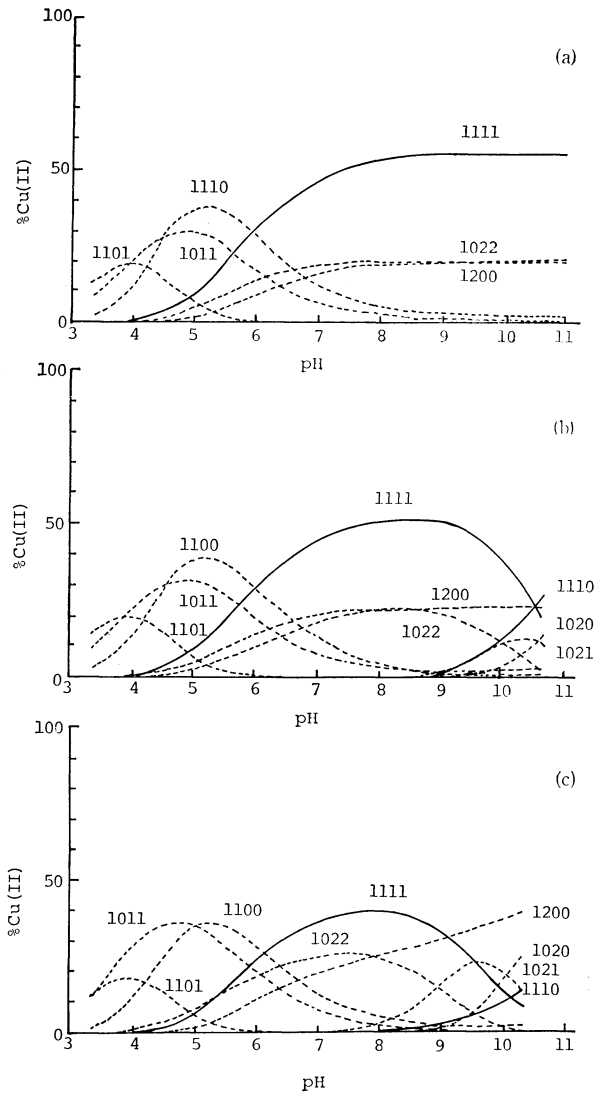



Fig. 5. Species distribution curves for the 1:1:1 Cu(II)–L-glu–L-B systems (25 °C; $I$=0.1 ($KNO_3$)).
Total concentrations: each ligand=0.002000 M; Cu(II)=0.002016 M.
(a): Cu(II)–L-glu–L-arg, (b): Cu(II)–L-glu–L-lys, (c): Cu(II)–L-glu–L-orn.

The only ternary species detected in the Cu(II)–L-A–L-arg systems is 1111 because of the tight association of the proton in the guanidinium group, whereas for the other systems both protonated (1111) and deprotonated (1110) species were found to be formed at neutral—alkaline pH. Figures 4 and 5 show the species distribution curves for the Cu(II)–L-asp–L-B and Cu(II)–L-glu–L-B systems, respectively. Although considerable amounts of the binary species are present in the latter systems as compared with the former, the ternary species 1111 are predominant at neutral pH in all the systems. We may take this as evidence supporting the previous assumption that the CD magnitude enhancements are ascribable to the ternary complexes (1111) with increased rigidity resulting from ligand-ligand interactions.[3]

A comparison of the stability constants with the statistically estimated values may offer another measure of preferential formation of the ternary species. The statistical values for species 1111 and 1110 are given by[16]

$$\log \beta_{1111(\mathrm{calcd})} = \frac{1}{2}(\log \beta_{1200} + \log \beta_{1022}) + \log 2, \quad (4)$$

$$\log \beta_{1110(\mathrm{calcd})} = \frac{1}{2}(\log \beta_{1200} + \log \beta_{1020}) + \log 2. \quad (5)$$

For most systems the experimental $\log \beta_{1111}$ values are greater than the calculated ones, substantiating the stabilization of species 1111, whereas the deprotonated species 1110 are usually less stable than expected from Eq. 5 (Table 5). The differences, $\log \beta_{1111}$—$\log \beta_{1110}$, are greater for Cu(L-A)(L-lys) and Cu(L-A)(L-orn) than for Cu(L-ala)(L-lys) and Cu(L-ala)(L-orn), probably reflecting the intramolecular electrostatic bonding.

*Stereoselectivity in Solution.* Optical resolution of

Table 5. Comparison of the stability constants with the statistically estimated values

| Ligand B | Ligand A | Stability $\log \beta_{1111}$ | $\log \beta_{1111(calcd)}$ [a] | $\log \beta_{1110}$ | $\log \beta_{1110(calcd)}$ [b] | $\log \beta_{1111} - \log \beta_{1110}$ |
|---|---|---|---|---|---|---|
| L-arg | L-asp | 27.43 | 27.18 | — | — | — |
| L-arg | L-glu | 26.61 | 26.66 | — | — | — |
| L-lys | L-asp | 26.32 | 26.04 | 15.82 | 15.76 | 10.50 |
| L-lys | L-glu | 25.60 | 25.52 | 15.08 | 15.24 | 10.52 |
| L-lys | L-ala | 25.52 | 25.58 | 15.59 | 15.30 | 9.93 |
| L-orn | L-asp | 25.65 | 25.55 | 15.29 | 16.08 | 10.36 |
| L-orn | L-glu | 24.93 | 25.03 | 14.68 | 15.56 | 10.25 |
| L-orn | L-ala | 24.86 | 25.09 | 15.37 | 15.61 | 9.49 |

a) Calculated according to Eq. 4. b) Calculated according to Eq. 5.

racemic A and B has established that the systems Cu(II)–DL-A–L-B and Cu(II)–L-A–DL-B preferentially give the *meso* complexes as crystals incorporating the D-enantiomers of A and B with various optical purities, respectively.[5] We see from Table 4 that the diastercomers, which may exist as geometric isomers (Scheme 1), have virtually the same stability constants and that no stereoselectivity is detected at least in the solution with $I=0.1$. Therefore, the optical resolution may be primarily due to the solubility difference and not to the stability difference between the diastereomers in solution under the conditions used.[5]

*Effects of Ionic Strength on Ternary Complex Formation.* In the Cu(II)–L-glu–L-lys system the stability constants for the ternary species 1111 and 1110 at lower ionic strength (0.03) were found to be higher than those at $I=0.1$ (Table 4), the increments being 0.32 for 1111 and 0.77 for 1110. The diastereomeric system Cu(II)–D-glu–L-lys exhibited the same stability increase at $I=0.03$. A similar observation was made by Nagypál *et al.*,[17] who found that the stability difference between experimental and statistical values for the copper(II)–glycine–aspartic acid complex varies with the ionic strength because of the change in charge on complex formation. The distributions of the ternary species in the present system are more predominant at lower ionic strength regardless of the enantiomers of A and B used.[18] Since such a trend is also expected for the other ternary systems and the CD spectral magnitude decrease due to increased ionic strength[3a,3c] has confirmed its effect on the electrostatic ligand-ligand interactions, the above result indicates that the stabilities of the complexes in aqueous solution are enhanced as the ligand-ligand interactions are reinforced.

In a hydrophobic region, such as is found in biological systems, electrostatic interactions as well as hydrogen bonds are certainly of importance, and elucidation of the complex formation *in vivo* will require further information on such non-covalent interactions, which might be more effective in biofluids than in experimental aqueous media.

This work was supported in part by a grant from the Ministry of Education.

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