# Stability Enhancement and Circular Dichroism Spectral Anomaly as an Indication of Non-covalent Interactions in Histidine- and Tyrosine-containing Ternary Copper(II)–Amino Acid Systems

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## Abstract

Visible absorption and CD** spectral and potentiometric studies on the His- and Tyr-containing ternary copper(II) complexes Cu(A)(*L*-B), where A refers to *L*-His, *D*-His, or *L*-Tyr and B to Lys, Tyr, Trp, Phe, Ala, Val, Arg, Glu, Asn, Gln, Ser, or Thr, were made to study ligand-ligand interactions in the complexes. While the CD spectral magnitudes in the d–d region are additive in the absence of side chain interactions and can be estimated from the magnitudes for the ternary systems involving *DL*-A or *DL*-B, deviation from the additivity was observed for Cu(*L*-His)(*L*-B) (B = LysH, Tyr, Trp, or Phe) and Cu(*L*-Tyr)(*L*-Trp). From the stability constants determined at 25 °C and $I$ = 0.1 M ($KNO_3$), the equilibrium constants, $K$, for the following hypothetical equilibria were calculated to be large (0.14–0.60) for formation of Cu(*L*-/*D*-His)(*L*-B) (B = Tyr or Trp) and Cu(*D*-His)(*L*-Phe) with Cu(en)(*L*-Ala) as standard:

$$\text{Cu(A)(}L\text{-Ala)} + \text{Cu(en)(}L\text{-B)} \overset{K}{\rightleftharpoons} \text{Cu(A)(}L\text{-B)} + \text{Cu(en)(}L\text{-Ala)}$$

The positive values indicate the stabilization due to the stacking between the imidazole ring of His and the aromatic side chain of *L*-B. Solvent dependence of the CD spectra for Cu(*L*-His)(*L*-LysH) and Cu(*L*-His)(*L*-Trp) further supported the existence of the intramolecular electrostatic and hydrophobic interactions.

## Introduction

A number of biological processes require non-covalent interactions between molecules or side chain groups of macromolecules for specificity and efficiency [1–3]. For systems involving transition metal complexes, the interactions may be ligand–ligand interactions around the central metal ion which places the interacting groups in appropriate positions. In addition, metal ions exert electronic effects on the coordinated ligands and make direct or through-metal $\pi$-donor–$\pi$-acceptor interactions possible, giving rise to ligand discrimination [4]. In view of the importance of non-covalent interactions in biological systems such as those evidenced by the X-ray crystal structure analysis of the carboxypeptidase A–substrate complex [5], we have studied their existence in metal complexes and the properties associated with them. Based on the CD spectral magnitude anomaly and NMR spectra, we revealed the electrostatic interactions and hydrogen bonds in ternary amino acid complexes of copper(II) [6–8] and palladium(II) [9–12] where the CD magnitudes, normally an additive function of the magnitudes for the component binary complexes, were found to become anomalous when the side chains interact with each other electrostatically [6–9] or through hydrogen bonding [12].

Ligand–ligand interactions may be further considered as models for the processes of molecular recognition by proteins, such as binding of peptide hormones by their receptors, irrespective of the essentiality of metal ions. Since His and Tyr offer aromatic rings and carboxyl groups that are expected to be involved in the interactions with the other amino acid side groups as well as metal ions, we investigated the possibility of non-covalent interactions in the ternary copper(II) systems with His or Tyr (referred to as A)

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**Following abbreviations were used throughout: CD, circular dichroism; His, histidinate; Tyr, tyrosinate; $TyrO^-$, Tyr with the deprotonated phenol OH group; Lys, lysinate; LysH, monoprotonated Lys; Trp, tryptophanate; Phe, phenylalaninate; Ala, alaninate; Val, valinate; Arg, argininate; Glu, glutamate; Asn, asparaginate; Gln, glutaminate; Ser, serinate; Thr, threoninate; Gly, glycinate; en, ethylenediamine; H, proton. For simplicity, Tyr and Arg refer to the monoprotonated forms with a proton attached to the phenol OH and the guanidinium group, respectively, and LysH is protonated at the $\epsilon$-amino group.

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TABLE I. Absorption and CD Spectral Data for Cu(*L*-His)(*L*-B) Systems in $H_2O$ and 50% Dioxane–$H_2O$.

| *L*-B | $H_2O$ | | | | | | | | 50% dioxane–$H_2O$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH | Absorption | | CD | | | | | pH | Absorption | | CD | | | | |
| | | $\lambda_{max}$ | $\epsilon$ | $\Delta\epsilon_{max}$ | $\Delta\epsilon_{obs}$ | $\Delta\epsilon_{calcd}$[a] | $\Delta\Delta\epsilon$[b] | $\Delta\Delta\epsilon_{max}$[c] | | $\lambda_{max}$ | $\epsilon$ | $\Delta\epsilon_{max}$ | $\Delta\epsilon_{obs}$ | $\Delta\epsilon_{calcd}$[a] | $\Delta\Delta\epsilon$[b] | $\Delta\Delta\epsilon_{max}$[c] |
| Ala | 6.9 | 606 | 57 | 620 | 0.24 | 0.25 | −0.01 | −0.01 | | | | | | | | |
| Val | 6.6 | 609 | 57 | 626 | 0.21 | 0.21 | 0.00 | −0.01 | 5.7 | 613 | 63 | 528 | −0.06 | −0.06 | 0.00 | −0.01 |
| | | | | | | | | | | | | 650 | 0.13 | 0.14 | −0.01 | |
| Ser | 6.6 | 610 | 55 | 628 | 0.21 | 0.21 | 0.00 | −0.01 | 6.0 | 614 | 59 | 522 | −0.03 | −0.03 | 0.00 | 0.00 |
| | | | | | | | | | | | | 640 | 0.18 | 0.18 | 0.00 | |
| Thr | 6.5 | 610 | 57 | 622 | 0.21 | 0.21 | 0.00 | 0.01 | 5.7 | 610 | 60 | 530 | −0.04 | −0.04 | 0.00 | 0.00 |
| | | | | | | | | | | | | 640 | 0.18 | 0.18 | 0.00 | |
| Asn | 6.6 | 609 | 55 | 620 | 0.28 | 0.28 | 0.00 | −0.01 | 5.9 | 614 | 59 | 637 | 0.24 | 0.25 | −0.01 | −0.01 |
| Gln | 6.6 | 612 | 59 | 620 | 0.24 | 0.24 | 0.00 | 0.00 | 5.9 | 607 | 62 | 500 | −0.02 | −0.02 | 0.00 | −0.01 |
| | | | | | | | | | | | | 636 | 0.20 | 0.21 | −0.01 | |
| Glu | 6.9 | 609 | 58 | 618 | 0.26 | 0.26 | 0.00 | 0.01 | | | | | | | | |
| Arg | 6.6 | 600 | 72 | 616 | 0.22 | 0.24 | −0.02 | −0.02 | 6.3 | 608 | 60 | 545 | −0.11 | −0.12 | 0.01 | ±0.01 |
| | | | | | | | | | | | | 665 | 0.16 | 0.16 | 0.00 | |
| LysH | 6.8 | 600 | 63 | 622 | 0.22 | 0.25 | −0.03 | −0.03 | 6.1 | 606 | 62 | 530 | −0.04 | −0.05 | 0.01 | 0.01 |
| | | | | | | | | | | | | 640 | 0.20 | 0.19 | 0.01 | |
| Lys | 9.9 | | | 635 | 0.24 | 0.24 | 0.00 | −0.01 | 9.8 | 602 | 65 | 520 | −0.04 | −0.04 | 0.00 | −0.01 |
| | | | | | | | | | | | | 636 | 0.20 | 0.21 | −0.01 | |
| Phe | 6.6 | 605 | 71 | 550 | −0.16 | −0.17 | 0.01 | 0.04 | 6.3 | 611 | 63 | 550 | −0.13 | −0.14 | 0.01 | 0.02 |
| | | | | 700 | 0.08 | 0.07 | 0.01 | | | | | 674 | 0.10 | 0.09 | 0.01 | |
| Tyr | 6.3 | 607 | 61 | 574 | −0.30 | −0.31 | 0.01 | 0.06 | 6.1 | 606 | 65 | 556 | −0.19 | −0.20 | 0.01 | 0.02 |
| | | | | 700[d] | 0.06 | 0.04 | 0.02 | | | | | 700[d] | 0.10 | 0.08 | 0.02 | |
| $TyrO^-$ | 9.4 | 600 | 63 | 553 | −0.23 | −0.23 | 0.00 | 0.04 | 9.1 | 610 | 67 | 556 | −0.20 | −0.21 | 0.01 | 0.01 |
| | | | | 700[d] | 0.09 | 0.09 | 0.00 | | | | | 700[d] | 0.09 | 0.08 | 0.01 | |
| Trp | 5.9 | 610 | 65 | 568 | −0.61 | −0.55 | −0.06 | −0.10 | 6.1 | 614 | 66 | 561 | −0.33 | −0.33 | 0.00 | −0.02 |
| | | | | 700 | 0.05 | 0.07 | −0.02 | | | | | 700 | 0.09 | 0.10 | −0.01 | |

[a]Calculated according to eqn. 3. [b]$\Delta\Delta\epsilon = \Delta\epsilon_{obs} - \Delta\epsilon_{calcd}$ at the maximum wavelength. [c]Maximum value of $\Delta\Delta\epsilon$ in the range 450–700 nm. [d]The maximum wavelength is at >700 nm.

TABLE II. Absorption and CD Spectral Data for Cu(*L*-Tyr or *L*-TyrO$^-$)(*L*-B) Systems.

| *L*-B | *L*-Tyr | | | | | | | | *L*-TyrO$^-$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | pH | Absorption | | CD | | | | | pH | Absorption | | CD | | | | |
| | | $\lambda_{max}$ | $\epsilon$ | $\Delta\epsilon_{max}$ | $\Delta\epsilon_{obs}$ | $\Delta\epsilon_{calcd}$[a] | $\Delta\Delta\epsilon$[b] | $\Delta\Delta\epsilon_{max}$[c] | | $\lambda_{max}$ | $\epsilon$ | $\Delta\epsilon_{max}$ | $\Delta\epsilon_{obs}$ | $\Delta\epsilon_{calcd}$[a] | $\Delta\Delta\epsilon$[b] | $\Delta\Delta\epsilon_{max}$[c] |
| Ala | 7.0 | 626 | 53 | 595 | −0.28 | −0.29 | 0.01 | 0.01 | 9.7 | 610 | 61 | 598 | −0.32 | −0.32 | 0.00 | −0.01 |
| Val | 6.1 | 624 | 56 | 594 | −0.33 | −0.33 | 0.00 | −0.01 | 9.9 | 624 | 61 | 590 | −0.37 | −0.37 | 0.00 | 0.01 |
| Glu | 7.8 | 608 | 44 | 590 | −0.31 | −0.32 | 0.01 | 0.01 | 10.2 | 611 | 55 | 590 | −0.42 | −0.42 | 0.00 | 0.02 |
| Arg | 8.3 | 612 | 51 | 592 | −0.32 | −0.31 | −0.01 | 0.01 | 10.0 | 611 | 55 | 590 | −0.42 | −0.42 | 0.00 | 0.02 |
| Phe | 6.9 | 620 | 55 | 593 | −0.42 | −0.42 | 0.00 | 0.02 | 10.2 | 622 | 58 | 600 | −0.45 | −0.45 | 0.00 | 0.02 |
| Trp | 6.2 | 602 | 50 | 600 | −0.48 | −0.53 | 0.05 | 0.05 | 9.0 | 606 | 56 | 600 | −0.55 | −0.60 | 0.05 | 0.05 |

[a]Calculated according to eqn. 3. [b]$\Delta\Delta\epsilon = \Delta\epsilon_{obs} - \Delta\epsilon_{calcd}$ at the maximum wavelength. [c]Maximum value of $\Delta\Delta\epsilon$ in the range 450–700 nm.

and an additional amino acid as a second ligand (B) by the CD spectra and the stability constants determined potentiometrically. The interactions in these systems may be of interest, because Tyr constitutes the essential N-terminus of the endogenous analgesic peptides (opioid peptides) [13] and His is involved as an essential component in peptide hormones thyrotropin releasing hormone (TRH), leuteinizing hormone releasing hormone (LH-RH), and adrenocorticotropic hormone (ACTH), all of which stimulate the brain activity [14]. The copper content is high in brain where both types of peptides are present [15]. This paper deals with the ligand-ligand interactions in Cu(*L*-/*D*-His)(*L*-B) and Cu(*L*-Tyr)-(*L*-B) as concluded from the CD magnitude anomaly and stability constants, and possible biological significance of the results.

## Experimental

### *Materials*

*L*- and *DL*-amino acids were purchased from Nakarai Chemicals. All the reagents used were of the highest grade available, and distilled and deionized water was used throughout.

### *Spectral Measurements*

Absorption spectra were recorded with a Union Giken SM-401 and a Hitachi 330 recording spectrophotometer, and CD spectra were obtained with a JASCO J-20 and a J-500C spectropolarimeter in a 1- or 5-cm path length quartz cell. Samples for the measurements were freshly prepared at various pH from the ligands and the stock solution of copper(II) perchlorate in the desired Cu(II)–ligand ratios, the concentrations of Cu(II) being 1–4 mM.

### *Determination of Stability Constants*

The stability constants for the ternary systems, Cu(II)–*L*-Tyr–*L*-B (B = Ala, Val, Arg, Glu, Phe, or Trp), Cu(II)–*L*-His–*L*-B (B = Ala or Tyr), Cu(II)–*D*-His–*L*-Tyr, and Cu(II)–en–*L*-B (B = Arg or Glu) were determined as described previously by pH titrations which were carried out at 25 °C and $I$ = 0.1 M ($KNO_3$) [16] for 1–2 mM solutions containing Cu(II), A, and B in the molar ratio of 1:1:1 and appropriate amounts of $HNO_3$. The stability constants $\beta_{pqrs}$ defined by eqn. 1 (charges are omitted) were calculated by using the program MINIQUAD [17]:

$$pCu + qA + rB + sH \rightleftharpoons Cu_pA_qB_rH_s$$

$$\beta_{pqrs} = \frac{[Cu_pA_qB_rH_s]}{[Cu]^p[A]^q[B]^r[H]^s} \quad (1)$$

where p, q, r, and s are the mol of Cu, A, B, and H, respectively, in the complex $Cu_pA_qB_rH_s$. The $pK_a$ values for A and B and the stability constants for the binary [18–21] and most of the His- and en-containing ternary systems [19, 22] were taken from the literature.

## Results and Discussion

### *Absorption and CD Spectra*

Absorption and CD spectral data for Cu(*L*-His)(*L*-B) and Cu(*L*-Tyr)(*L*-B) are summarized in Tables I and II, respectively. The maximum wavelengths in the d–d region of the absorption spectra in water are in the range 600–612 nm and 602–626 nm for *L*-His- and *L*-Tyr-containing systems, respectively. The CD spectra of the Cu(*L*-His)(*L*-B) systems show a positive peak when *L*-B is aliphatic and a negative peak when it is aromatic. For the ternary Cu(II)– and Pd(II)–amino acid systems without ligand–ligand interactions, the CD spectral magnitudes in the d–d region proved to be an additive function of the magnitudes of the component binary complexes [6–9, 12], and the magnitude ($\Delta\epsilon$) for Cu(*L*-X)(*L*-Y), where X and Y are amino acids coordinating in the glycine-like mode, is given by the magnitudes for binary complexes Cu(*L*-X)$_2$ and Cu(*L*-Y)$_2$ or those for the ternary systems with Gly according to eqn. 2:

$$\Delta\epsilon_{Cu(L\text{-}X)(L\text{-}Y)} = \tfrac{1}{2}\{\Delta\epsilon_{Cu(L\text{-}X)_2} + \Delta\epsilon_{Cu(L\text{-}Y)_2}\} = \Delta\epsilon_{Cu(L\text{-}X)(Gly)} + \Delta\epsilon_{Cu(L\text{-}Y)(Gly)} \quad (2)$$

This is in accord with the additivity in Cu(II)-, Ni(II)-, and Pd(II)-peptide complexes [23]. Serious deviations from the magnitude additivity have been observed for the systems with electrostatic interactions between the side chains of X and Y and concluded to serve as a measure of ligand–ligand interactions [6–9, 12]. For the systems involving His and/or an aromatic amino acid, however, eqn. 2 does not hold because of the coordination by the imidazole group of His and probably the Cu(II)–aromatic ring interaction [24, 25], so that eqn. 3 which avoids the change in the ligand field is more properly employed in the present cases [12]:

$$\Delta\epsilon_{Cu(L\text{-}A)(L\text{-}B)} = \Delta\epsilon_{Cu(DL\text{-}A)(L\text{-}B)} + \Delta\epsilon_{Cu(L\text{-}A)(DL\text{-}B)} \quad (3)$$

Figure 1 illustrates satisfactory fits between the observed and calculated curves in the visible region for the ternary systems Cu(*L*-His)(*L*-Val) and Cu(*L*-Tyr)(*L*-Val), the differences $|\Delta\Delta\epsilon|$ between the observed ($\Delta\epsilon_{obs}$) and calculated ($\Delta\epsilon_{calcd}$) magnitudes, $|\Delta\epsilon_{obs} - \Delta\epsilon_{calcd}|$ being $\leq 0.01$. The magnitudes for Cu(*L*-His)(*L*-B) have also been found to be additive

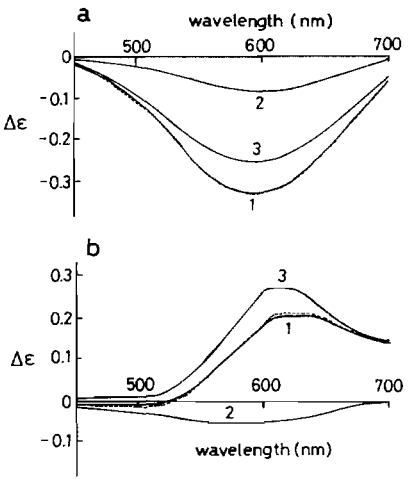


Fig. 1. CD magnitude additivity in Cu(Tyr)(Val) (a) and Cu(His)(Val) (b) systems at pH 6.1 and 6.6, respectively. Curve: 1, Cu(*L*-A)(*L*-B); 2, Cu(*DL*-A)(*L*-B); 3, Cu(*L*-A)(*DL*-B). The dotted line was calculated according to eqn. 3.

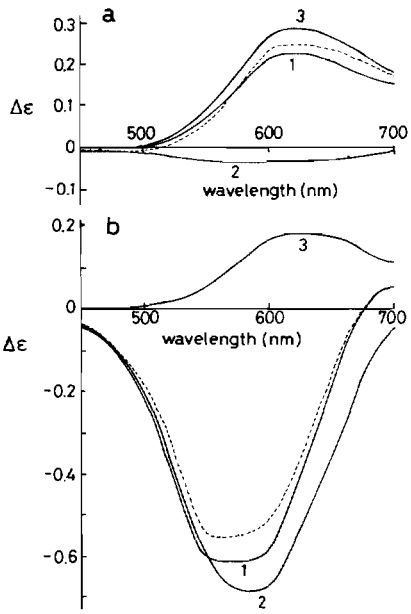


Fig. 2. CD magnitude additivity in Cu(His)(LysH) (a) and Cu(His)(Trp) (b) systems at pH 6.8 and 5.9, respectively. Curve: 1, Cu(*L*-A)(*L*-B); 2, Cu(*DL*-A)(*L*-B); 3, Cu(*L*-A)(*DL*-B). The dotted line was calculated according to eqn. 3.

when B is Ser, Thr, Asn or Gln (Table I). For the systems with B = LysH, Phe, Trp and Tyr, definite deviations from the calculated values ($\geq 0.03$) were detected, and somewhat smaller deviations were found for B = Arg and $TyrO^-$ (Table I and Fig. 2). CD magnitude anomalies for the Tyr-containing systems were observed only for Cu(*L*-Tyr)(*L*-Trp) and Cu(*L*-$TyrO^-$)(*L*-Trp) (Table II).

TABLE III. Stability Constants log $\beta_{pqrs}$ for Ternary Cu(A)(*L*-B) Systems at 25 °C and $I$ = 0.1 M ($KNO_3$).[a]

| *L*-B | species | A | | | |
|---|---|---|---|---|---|
| | | *L*-Tyr | *L*-His | *D*-His | en |
| Ala | 1111 | 25.583(2) | 21.77(1) | | |
| | 1110 | 16.052(2) | 17.750(1) | | 17.949[b] |
| Val | 1111 | 25.490(3) | | | |
| | 1110 | 15.901(3) | 17.603[c] | 17.546[c] | 17.726[b] |
| Arg | 1112 | 37.018(4) | | | |
| | 1111 | 27.608(4) | 29.250[c] | 29.126[c] | 29.398(4) |
| Lys | 1111 | | 27.883[c] | 27.78[c] | 27.915(6) |
| | 1110 | | 17.12[c] | 17.12[c] | 17.629(8) |
| Glu | 1112 | 30.56(1) | 26.68[c] | 26.68[c] | |
| | 1111 | 25.996(4) | 22.697[c] | 22.697[c] | 23.267(9) |
| | 1110 | 16.369(5) | 17.864[c] | 17.864[c] | 18.317(2) |
| Phe | 1111 | 25.446(1) | 21.44[c] | 21.45[c] | |
| | 1110 | 15.861(2) | 17.504[c] | 17.699[c] | 17.746[b] |
| Tyr | 1112 | | 31.72(3) | 31.44(5) | |
| | 1111 | | 27.735(3) | 27.820(4) | 27.772[b] |
| | 1110 | | 17.991(4) | 17.751(5) | 18.462[b] |
| Trp | 1111 | 25.608(3) | | | |
| | 1110 | 16.090(4) | 18.003[c] | 18.475[c] | 18.078[b] |

[a]Values in parentheses denote estimated standard deviations. The $pK_a$ values and the stability constants for the binary complexes were taken from ref. 20 (Ala), ref. 19 (en, Val, Glu, Phe, Trp and His), ref. 18 (Arg and Lys), and ref. 21 (Tyr). [b]Taken from ref. 22. [c]Taken from ref. 19.

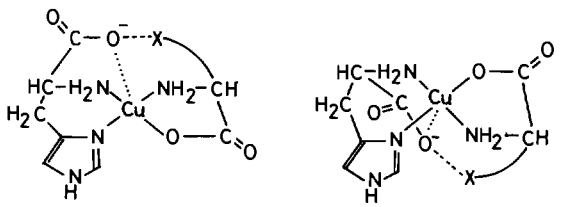


Fig. 3. Proposed structures of Cu(*L*-/*D*-His)(*L*-B) complexes involving electrostatic and hydrogen bonds [19, 25, 26].

Electrostatic interactions and hydrogen bonding between ligands in Cu(*L*-His)(*L*-B) have been proposed to occur through the axially coordinated carboxylate oxygen and the charged side chains (B = Arg or LusH) [19, 26] or the polar groups (B = Ser, Thr, Asn, or Gln) [25, 27] (Fig. 3). The CD anomaly observed for Cu(*L*-His)(*L*-LysH) (Table I) corresponds to the interaction proposed on the basis of the stability constants $\beta_{1111}$ (Fig. 3), but for the latter cases hydrogen bonding, which has been established in the corresponding Pd(II) complexes by NMR studies [10, 12], appears to have no effect on the CD magnitudes under the conditions employed. *Cis–trans* isomerism, expected from the intramolecular interaction in Cu(*L*-His)(*L*-Asn) and Cu(*L*-His)(*D*-Asn), is supported by the infrared spectra [28].

*Stability Constants*

The stability constants for the ternary Cu(II)–ligand systems are shown in Table III. Probably because of the aromatic ring stacking the meso complexes Cu(*D*-His)(*L*-B) with B = Phe, Tyr or Trp are preferentially formed relative to the active ones. For the systems Cu(*L*-/*D*-His)(*L*-Tyr) deprotonation of the phenol OH group to give *L*-TyrO$^-$ reverses the stability order, making the active form Cu(*L*-His)(*L*-TyrO$^-$) more stable than the *meso* form. The calculated percentage distributions of the ternary systems, as shown in Fig. 4, indicate that at pH 6–7 the ternary complexes Cu(*L*-/*D*-His)(*L*-B) account for 75–88% (B = Phe, Tyr or Trp), 71–80% (B = Arg or LysH), and 71–74% (B = Val) of the total Cu(II). The results confirm that the observed spectral properties at neutral pH are mainly ascribed to the ternary species.

We may evaluate the stability enhancement due to ligand–ligand interactions in the Cu(*L*-/*D* His)-(*L*-B) systems by comparing the stability constants with the constant for Cu(en)(*L*-Ala) as a standard where no interactions are possible. Combining the model equilibria expressed by eqns. 4 and 5, we obtain a hypothetical equilibrium (eqn. 6) and its equilibrium constant (eqn. 7):

TABLE IV. Log $K$ Values for Cu($L$-/$D$-His)($L$-B) Systems.

| $L$-B | species | log $K$ | | CD additivity |
|---|---|---|---|---|
| | | $L$-His | $D$-His | |
| Ala | 1110 | 0.00 | 0.00[a] | + |
| Val | 1110 | 0.09 | 0.03 | + |
| Arg | 1111 | 0.06 | −0.07 | ± |
| LysH | 1111 | 0.18 | 0.08 | − |
| Lys | 1110 | −0.31 | −0.31 | + |
| Glu | 1110 | −0.25 | −0.25 | + |
| Phe | 1110 | −0.04 | 0.16 | − |
| Tyr | 1111 | 0.16 | 0.25 | − |
| TyrO$^-$ | 1110 | −0.27 | −0.51 | ± |
| Trp | 1110 | 0.14 | 0.60 | − |

[a]The value for Cu($D$-His)($L$-Ala) was assumed to be equal to that of Cu($L$-His)($L$-Ala).

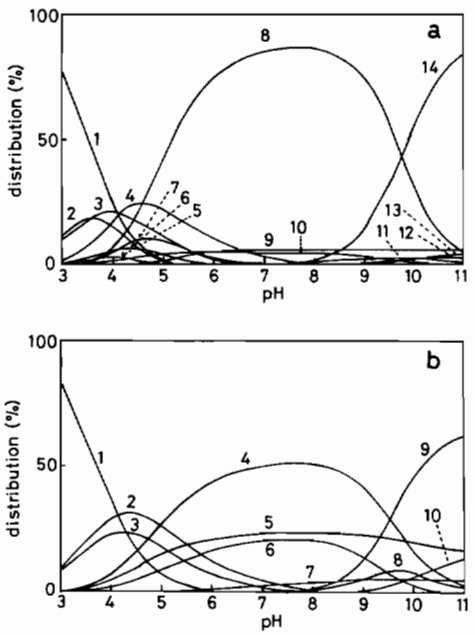


Fig. 4. Species distributions in the ternary Cu($L$-A)($L$-B) systems as a function of pH (25 °C; $I$ = 0.1 M ($KNO_3$)). a, 1:1:1 Cu(II)–$L$-Tyr–$L$-His (2 mM); b, 1:1:1 Cu(II)–$L$-Tyr–$L$-Trp (2 mM). Curves correspond to the following species (pqrs): (a) 1, 1000; 2, 1011; 3, 1101; 4, 1010; 5, 1022; 6, 1112; 7, 1021; 8, 1111; 9, 1020; 10, 1202; 11, 1100; 12, 1201; 13, 1200; 14, 1110; (b) 1, 1000; 2, 1101; 3, 1010; 4, 1111; 5, 1020; 6, 1202; 7, 1100; 8, 1201; 9, 1110; 10, 1200.

$$\text{Cu}(L\text{-}/D\text{-His})(L\text{-Ala}) + \text{Cu}(L\text{-B}) \rightleftharpoons \text{Cu}(L\text{-}/D\text{-His})(L\text{-B}) + \text{Cu}(L\text{-Ala}) \quad (4)$$

$$\text{Cu(en)}(L\text{-B}) + \text{Cu}(L\text{-Ala}) \rightleftharpoons \text{Cu(en)}(L\text{-Ala}) + \text{Cu}(L\text{-B}) \quad (5)$$

TABLE V. Log $K$ Values for Cu($L$-Tyr or $L$-TyrO$^-$)–($L$-B) Systems.

| $L$-B | $L$-Tyr | | $L$-TyrO$^-$ | |
|---|---|---|---|---|
| | log $K$ | CD additivity | log $K$ | CD additivity |
| Ala | 0.00 | + | 0.00 | + |
| Val | 0.14 | + | 0.13 | + |
| Arg | 0.01 | + | 0.05 | + |
| Glu | 0.08 | + | 0.11 | + |
| Phe | 0.07 | + | 0.02 | + |
| Trp | −0.09 | − | −0.08 | − |

$$\text{Cu}(L\text{-}/D\text{-His})(L\text{-Ala}) + \text{Cu(en)}(L\text{-B}) \overset{K}{\rightleftharpoons} \text{Cu}(L\text{-}/D\text{-His})(L\text{-B}) + \text{Cu(en)}(L\text{-Ala}) \quad (6)$$

$$\log K = \log \beta_{\text{Cu}(L\text{-}/D\text{-His})(L\text{-B})} + \log \beta_{\text{Cu(en)}(L\text{-Ala})} - \log \beta_{\text{Cu}(L\text{-}/D\text{-His})(L\text{-Ala})} - \log \beta_{\text{Cu(en)}(L\text{-B})} \quad (7)$$

where $\beta_{\text{Cu}(L\text{-}/D\text{-His})(L\text{-B})}$ *etc.* refer to $\beta_{1110}$ or $\beta_{1111}$. The equation for the Tyr-containing systems is obtained in the same way:

$$\text{Cu}(L\text{-Tyr})(L\text{-Ala}) + \text{Cu(en)}(L\text{-B}) \overset{K}{\rightleftharpoons} \text{Cu}(L\text{-Tyr})(L\text{-B}) + \text{Cu(en)}(L\text{-B}) \quad (8)$$

Tables IV and V list the log $K$ values for Cu($L$-/$D$-His)($L$-B) and Cu($L$-Tyr)($L$-B), respectively. In accordance with the earlier observation [19, 26], the value for Cu($L$-His)($L$-LysH) is positive while the values for Cu($L$-His)($L$-Lys) and Cu($L$-His)($L$-Glu) are negative, indicating that the stabilization of Cu($L$-His)($L$-LysH) is due to the electrostatic interaction shown in Fig. 3. The Cu($L$-/$D$-His)($L$-B) systems with B = Phe, Tyr or Trp also have enhanced log $K$ values, which may reflect the stacking interaction between the imidazole group of His and the aromatic side chain of $L$-B. Interestingly, dissociation of the OH group of Tyr drastically reduces the log $K$ values to −0.27 for $L$-His and −0.51 for $D$-His. The hydrogen bonding inferred for the $L$-His-containing ternary systems with B = Ser, Thr, homoserinate, Asn, Gln, or citrullinate [25] has previously been found to cause no stereoselectivity in 20% aqueous dioxane [29].

### *Intramolecular Aromatic Ring Stacking as Evidenced by CD Spectral Magnitudes and Stability Constants*

CD magnitude anomaly and enhanced log $K$ values point to the stacking in Cu($L$-/$D$-His)($L$-B) between the imidazole ring and the aromatic ring of $L$-B (B = Phe, Tyr or Trp) (Fig. 5). The fact that the log $K$ values increase in the order of B, Phe <

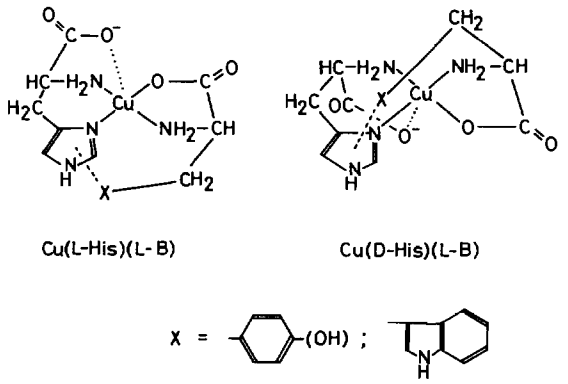


Fig. 5. Proposed structures of Cu(*L*-/*D*-His)(*L*-B) complexes involving aromatic ring stacking.

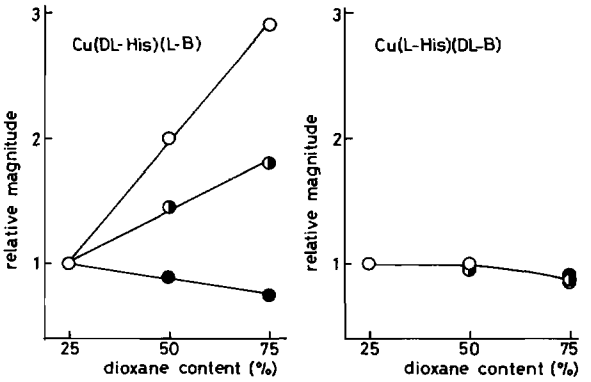


Fig. 6. Solvent dependences of CD magnitudes for Cu(*DL*-His)(*L*-B) and Cu(*L*-His)(*DL*-B) systems. B: LysH (○); Val (●); Trp (●). The relative magnitude is based on the CD magnitude at 25% dioxane–$H_2O$.

Tyr < Trp and decrease upon dissociation of the phenol group by 0.43 and 0.76 log unit for the *L*-His- and *D*-His-containing systems, respectively, supports the aromatic ring stacking in the ternary complexes, because it manifests the hydrophobic character of the interaction and the resulting complex stabilization. The stacking may be compatible with the possible Cu(II)–aromatic ring interaction that has been inferred from the X-ray crystal structure analyses of Cu(*L*-Tyr)$_2$ [30], Cu(Gly·*L*-Trp) [31] (Gly·*L*-Trp = glycyl-*L*-tryptophanate) etc. [32], although its contribution to log *K* is cancelled in the formulation of log *K*.

For the Cu(*L*-Tyr)(*L*-Trp) system the log *K* value is negative, probably because of the steric hindrance due to the two side chain aromatic rings. Since the p$K_a$ value (9.52) of the phenol group in Cu(*L*-Tyr)(*L*-Trp) is equal to that in Cu(*L*-Tyr)(*L*-Ala), no stacking is inferred for this system.

Further evidence supporting the existence of the electrostatic and hydrophobic interactions as shown in Figs. 3 and 5 is furnished by the CD spectra of Cu(*DL*-His)(*L*-LysH) and Cu(*DL*-His)(*L*-Trp) in solvents with different polarity (Fig. 6). With the increase of the dioxane content the CD magnitude enhancement is observed for both Cu(*DL*-His)(*L*-LysH) and Cu(*DL*-His)(*L*-Val), but the enhancement as expressed in relative magnitude (relative to that in 25% aqueous dioxane) is greater for the former, indicating that electrostatic interactions become more effective in solvents with lower polarity. On the other hand, the relative magnitude for Cu(*DL*-His)(*L*-Trp) decreases from unity with the dioxane content, interpreted as being due to weakened aromatic ring stacking. This trend is already apparent from Table I, showing the solvent dependence of the magnitude additivity. Interestingly, the CD magnitudes due to *L*-His in the ternary systems with *DL*-LysH and *DL*-Trp do not change with the dioxane content; this may be taken to show that the conformation and mode of coordination of His remain unaffected by the solvent polarity.

The hitherto-determined structures of Cu(*L*-His)(*L*-B) (B = Thr [33], Asn [34], Ala [35] or Ser [36]) in the solid state have a *cis* configuration in the coordination plane with the carboxylate oxygen of His apically coordinated to Cu(II) except in the latter two. Considering that the log *K* values are larger for the *meso* complexes, the *cis* configuration would favor intramolecular stacking, because the carboxyl group is then located on the opposite side of the plane and does not interfere with the approach of the aromatic ring to the imidazole of coordinated His (Fig. 5).

*Possible Biological Significance*

The present study on the ligand–ligand interactions in the ternary Cu(II)–amino acid complexes shows that the imidazole ring of His participates in stacking with the aromatic side chains of Phe, Tyr and Trp. The stacking may require a metal ion such as Cu(II) whose template effect and affinity for aromatic rings contribute to the close contact between two rings. On the other hand, the phenol moiety of Tyr in the ternary Cu(II) systems appears to interact effectively with the imidazole ring only, although this does not exclude its interaction with the aromatic rings of Phe and Trp. In this connection the receptor site of opioid peptides such as enkephalin, whose N-terminal amino acid is Tyr, has been reported to involve essential imidazole for the physiological activity [37], which may suggest that stacking possibly in the presence of Cu(II) is important in the peptide-receptor bonding. Deprotonation of the OH group or its modulation by phosphorylation and sulfation weakens stacking, which might correspond to release of opioid peptides. Studies on stacking interactions in model complexes along this line are in progress in our laboratory. The presence of an

anomalously high concentration of copper in the brain [14] suggests the possibility that Cu(II) and its peptide complexes play a role in the opioid peptide activity.

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