Histidine-Containing Ternary Amino Acid–Copper(II) Complexes. Syntheses and Properties

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Abstract: Ternary copper(II) complexes Cu(His)(AA), where His refers to L- or D-histidine and AA to L- or D-asparagine (L- or D-Asn), L-glutamine (L-Gln), L-serine (L-Ser), L-homoserine, or L-citrulline (L-Cit), have been isolated as crystals. The Cu(II)–L-His–L-AA systems in the visible region almost invariably exhibited absorption peaks at 610–620 nm and positive circular dichroism (CD) extrema at 620–630 nm at pH 7–8, suggesting similar coordinating groups in the copper(II) coordination plane. On the basis of the difference in crystallization between the diastereomers Cu(L-Asn)(L-His) and Cu(D-Asn)(L-His) and Cu(L-Cit)(L-His) and Cu(L-Cit)(D-His), optical resolution of DL-histidine has been performed via formation of the ternary complexes, Cu(L-Asn)(His) and Cu(L-Cit)(His), which were shown to have incorporated L-histidine with optical purities as high as 98 and 78%, respectively, by the calibration curves established by the CD spectral magnitudes. The stability constants (log β values) for the ternary complexes with L-Asn, L-Gln, L-Ser, and L-Thr have been determined to be in the range 17.0–17.1 by potentiometric titrations at 25 °C in 0.1 M KNO_3. Facile isolation of the ternary complexes and successful optical resolution of DL-histidine are interpreted in terms of the solubility differences and the intramolecular hydrogen bond inferred to be formed between the carboxylate oxygen of histidine and the amide or the hydroxyl group present in the side chain of AA. A plausible structure of the ternary complexes in solution has been proposed by assuming terdentate histidine and this intramolecular interaction.

Histidine (His) is perhaps the most frequently found and most important metal-binding site in biological systems. Its participation in complex formation has been established by the X-ray crystal structure analysis of carboxypeptidase A,[2] carbonic anhydrase B[3a] and C,[3b] thermolysin,[4] plastocyanin,[5] superoxide dismutase,[6] etc., and has been proposed for many other enzymes and metalloproteins. The modes of coordination by histidine at the active sites have thus attracted much attention, and there have been reported a number of investigations aimed at elucidating the structures and functions of histidine-containing complexes formed in biological systems from the properties of model complexes.[7] An unequivocal answer to the structural problems has been provided by the X-ray analysis by Freeman et al.[8] of a ternary copper(II) complex with L-threonine (L-Thr), Cu(L-His)(L-Thr), where the two amino groups occupy the cis positions and histidine serves as a terdentate ligand coordinating through the amino and the imidazole nitrogen in the square plane and the carboxyl oxygen in the apical position. Another interesting ternary complex, Cu(L-His)(D-His), has been disclosed very recently by Camerman et al.[9] to involve both histidines coordinating as bidentate ligands through the amino and the imidazole nitrogen in a trans arrangement in the square plane with two water molecules in the apical positions. In spite of increasing information from these X-ray analyses as well as recent detailed potentiometric,[10-16] spectroscopic,[12,17-24] and calorimetric[12,15,25] studies, however, there still remain uncertainties about the structures of histidine-containing binary and ternary complexes in solution, and synthesis of ternary complexes with the other α-amino acids have hitherto been unsuccessful, making the structure determination more difficult.

In addition to the histidyl residue at the metal binding site in macromolecules, histidine itself is reported to be involved in copper(II) transport in blood.[26-30] From a comparative ^{64}Cu tracer study of the effects of combinations of amino acids on the copper(II) complex formation in predialyzed human blood serum, Neumann and Sass-Kortsak[28] concluded that histidine forms low-molecular-weight ternary copper(II) complexes preferentially with asparagine (Asn), glutamine (Gln), and threonine, and Sarkar and Kruck[26] actually detected Cu(L-His)(L-Thr) by thin layer chromatography using ^{64}Cu and ^{14}C as tracers. On the other hand, computer simulation studies on the solution equilibria in blood plasma[31-33] have shown that the most predominant copper(II) complex species present among low-molecular-weight biological ligands such as amino acids is the ternary complex containing histidine and cystine, which is followed by $Cu(His)_2$. However, the preferential formation of the mentioned ternary complexes and the discrepancy between the results of the tracer and the simulation studies seem to require an explanation, which neither the molecular structure of Cu(L-His)(L-Thr) in the solid state nor the solution equilibria have furnished.

In view of the importance of noncovalent interactions, such as hydrogen bonds and electrostatic interactions, as a key to the understanding of the specificity and high efficiency exhibited by enzymes,[34] we have been studying ternary amino acid–copper(II) complexes with intramolecular ligand–ligand interactions as models for the enzyme–metal–substrate complex formed in enzymatic reactions.[35] It appeared to us most intriguing that His, Asn, Gln, and Thr mentioned above have polar side groups that may form intramolecular hydrogen bonds under favorable conditions when each of the three pairs of amino acids, His-Asn, His-Gln, and His-Thr, is coordinated around copper(II) in a configuration as disclosed by X-ray analysis.[8] In this connection, we reported in a previous communication[36] that L-asparaginato-L-histidinatocopper(II) can be isolated in two modifications and that racemic histidine is resolved into enantiomers by highly selective incorporation of the L enantiomer into the ternary copper(II) complex with L-asparagine.

In a continued attempt at preparing histidine-containing complexes, we isolated as crystals a number of ternary copper(II) complexes with α-amino acids with a polar side chain (referred to as AA hereafter), such as glutamine, serine (Ser), and citrulline (Cit), and investigated the spectral behaviors, the solution equilibria, and the optical resolution of racemic histidine via ternary complex formation. The present paper describes the results of these experiments, the discussion of the structures of the complexes in solution, and the possibility of the intramolecular ligand–ligand interactions as a factor governing the preferential formation of certain ternary complexes.

Experimental Section

Materials. Analytical reagent grade L- and D-histidine, L- and D-asparagine hydrate, L-glutamine, L-threonine, L-serine, L-ho-

0002-7863/79/1501-4164$01.00/0

moserine (L-Hmser), L-citrulline, L-alanine (L-Ala), and L-valine (L-Val) were purchased from Nakarai Chemicals, Ltd. Ultrapure reagent grade L-histidine hydrochloride monohydrate, L-asparagine hydrate, L-glutamine, L-serine, and L-threonine obtained from Ajinomoto Chemical Co. were used for pH titrations and spectroscopic measurements. Water was distilled and deionized. All other chemicals used were of highest grade available.

Synthesis of Ternary Complexes. [Cu(L-Asn)(L-His)]·nH$_2$O (n = 0 and 4).[36] The complex was obtained from solutions containing equimolar amounts of L-histidine, L-asparagine, and $Cu(ClO_4)_2 \cdot 6H_2O$ at pH 7.5–8.0. Depending on the conditions used for crystallization, the complex was isolated in two modifications.[37] The tetrahydrate was obtained as blue needles from an aqueous solution. Anal. Calcd for $CuC_{10}H_{15}N_5O_5 \cdot 4H_2O$: C, 28.54; H, 5.51; N, 16.64. Found: C, 28.37; H, 5.52; N, 16.58.

Crystallization from 50% aqueous methanol or ethanol and drying in the open air gave the anhydrous complex as deep blue prismatic crystals.[37] Anal. Calcd for $CuC_{10}H_{15}N_5O_5$: C, 34.43; H, 4.33; N, 20.08. Found: C, 34.09; H, 4.57; N, 19.93.

[Cu(D-Asn)(L-His)]·nH$_2$O (n = 2 and 3). The meso complex was prepared in the manner described for Cu(L-Asn)(L-His) except that the molar ratio of Cu(II):D-Asn:L-His was kept at 1:0.9:1.3, which prevented the precipitation of less soluble Cu(D-Asn)$_2$. Crystallization from aqueous ethanol with a low and a high ethanol content gave the complex as a trihydrate and a dihydrate, respectively. Anal. Calcd for $CuC_{10}H_{15}H_5O_5 \cdot 2H_2O$: C, 31.21; H, 4.98; N, 18.20. Found: C, 31.20; H, 4.84; N, 18.33. Calcd for $CuC_{10}H_{15}N_5O_5 \cdot 3H_2O$: C, 29.81; H, 5.25; N, 17.39. Found: C, 29.50; H, 5.31; N, 17.49.

[Cu(L-His)(L-Ser)]·nH$_2$O (n = 1 and 4). L-Histidine (0.78 g, 5.0 mmol), L-serine (1.05 g, 10.0 mmol), and $Cu(ClO_4)_2 \cdot 6H_2O$ (1.85 g, 5.0 mmol) were dissolved in 30 mL of water, and the pH of the resulting solution was adjusted at 7.5. After addition of ethanol, the solution was kept in a refrigerator, when blue crystals separated. Recrystallization from 50% aqueous ethanol gave the tetrahydrate as blue crystals. Anal. Calcd for $CuC_9H_{14}N_4O_5 \cdot 4H_2O$: C, 27.45; H, 5.63; N, 14.23. Found: C, 27.60; H, 5.51; N, 14.35.

The monohydrate was obtained by drying the tetrahydrate in vacuo. Anal. Calcd for $CuC_9H_{14}N_4O_5 \cdot H_2O$: C, 31.81; H, 4.75; N, 16.49. Found: C, 31.71; H, 4.80; N, 16.54.

[Cu(L-His)(L-Hmser)]·3H$_2$O. L-Histidine (0.70 g, 4.5 mmol), L-homoserine (0.77 g, 6.5 mmol), and $Cu(ClO_4)_2 \cdot 6H_2O$ (1.85 g, 5.0 mmol) were dissolved in ca. 10 mL of water, and the crystals were obtained in the manner described above, washed with ethanol, and dried over $CaCl_2$. Blue needles were obtained. Anal. Calcd for $CuC_{10}H_{16}H_4O_5 \cdot 3H_2O$: C, 30.81; H, 5.69; N, 14.37. Found: C, 30.76; H, 5.73; N, 14.54.

[Cu(D-His)(L-Hmser)]·3H$_2$O. This was obtained as blue needles by the same method as described for the active complex. Anal. Calcd for $CuC_{10}H_{16}N_4O_5 \cdot 3H_2O$: C, 30.81; H, 5.69; N, 14.37. Found: C, 30.78; H, 5.64; N, 14.40.

[Cu(L-His)(L-Thr)]·2H$_2$O. This complex was prepared as blue crystals by the method described for Cu(L-His)(L-Ser). The crystal parameters[38] were the same as those reported by Freeman et al.[8] Anal. Calcd for $CuC_{10}H_{16}N_4O_5 \cdot 2H_2O$: C, 32.00; H, 5.43; N, 15.07. Found: C, 32.30; H, 5.42; N, 15.07.

[Cu(L-Gln)(D-His)]·2H$_2$O. D-Histidine (0.78 g, 5.0 mmol), L-glutamine (0.73 g, 5.0 mmol), and $Cu(ClO_4)_2 \cdot 6H_2O$ (1.85 g, 5.0 mmol) were dissolved in 30 mL of water at pH 9. The solution was then concentrated in vacuo at <50 °C, and the residue was washed with methanol to give blue crystals, which were filtered and recrystallized from aqueous methanol. As the isolated complex was found to contain one molecule of methanol, the crystals were dried in vacuo at 60 °C for 3 h and equilibrated with the moist air in a closed vessel to give the dihydrate of the complex. Anal. Calcd for $CuC_{11}H_{17}N_5O_5 \cdot 2H_2O$: C, 33.12; H, 5.31; N, 17.56. Found: C, 32.85; H, 4.95; N, 17.71.

[Cu(L-Cit)(L-His)]·2H$_2$O. L-Histidine (1.01 g, 6.5 mmol), L-citrulline (0.79 g, 4.5 mmol), and $Cu(ClO_4)_2 \cdot 6H_2O$ (1.85 g, 5.0 mmol) were dissolved in 20 mL of water at pH 7.5, and the complex was isolated as a blue, crystalline powder in the same manner as described for Cu(L-His)(L-Ser). Anal. Calcd for $CuC_{12}H_{20}N_6O_5 \cdot 2H_2O$: C, 33.68; H, 5.65; N, 19.64. Found: C, 33.56; H, 5.56; N, 19.62.

Instruments. Infrared spectra were recorded with the KBr disk method using a Hitachi 215 grating infrared spectrophotometer in the range 4000–650 cm^{-1} and a Hitachi EPI-L grating infrared spectrophotometer in the range 700–200 cm^{-1} in dry air. Absorption spectra in the visible region (400–800 nm) were measured with a Union Giken SM-401 high sensitivity recording spectrophotometer. Circular dichroism (CD) spectra were obtained in the range 350–800 nm with a JASCO MOE-1 spectropolarimeter. Optical rotations were measured at 589 nm with a Yanagimoto OR-10 polarimeter at 20 ± 0.1 °C. Measurements of pH were made with an Orion Research 801A 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 (pH 4.01, 6.86, and 9.18 at 25 °C).

Spectroscopic Measurements. The absorption and CD spectra were measured at room temperature for the systems involving Cu(II), His, and an amino acid in the ratios of 1:2:0 or 1:0:2 (binary systems) and 1:1:1 (ternary systems) at pH 4–10. The samples were prepared from 0.1 M stock solutions of amino acids and copper(II) perchlorate in water. The pH values were adjusted with aqueous sodium hydroxide (5%) and perchloric acid (5%) and checked after the measurements. The ionic strength (I) of the solutions was usually unadjusted (I = var), except for the cases where a constant ionic strength (I = 0.1 (KNO_3)) was required.

Potentiometric Titrations. Carbonate-free potassium hydroxide (0.1 M) was prepared according to the method of Armstrong,[39] standardized against potassium hydrogen phthalate, and stored under a nitrogen atmosphere. Copper(II) nitrate (0.01 M) was standardized by chelatometric titrations using EDTA standardized against zinc (JIS primary standard). For determination of the stability constants of ternary complexes, aqueous solutions of 1:1:1 copper(II)–histidine–AA were titrated with 0.1 M potassium hydroxide at 25 ± 0.05 °C under a nitrogen atmosphere, the initial total concentration of copper(II) being 2×10^{-3} M. All the titrations were performed in 0.1 M potassium nitrate (I = 0.1). Reproducibility of data was checked by duplicate runs. In order to get analytical concentrations of hydrogen and hydroxide ions, we determined the apparent ion product of water (pK_w') and the ratio of $10^{-pH}/10^{-\log[H^+]}$ to be 13.97 and 0.85, respectively, by titrating dilute nitric acid with 0.1 M potassium hydroxide at I = 0.1.

Optical Resolution of Racemic Histidine via Complex Formation. A. Optical Resolution via Cu(L-Asn)(His). Isolation of the complex in two modifications from 1:1:1.5 Cu(II)–L-Asn–DL-His systems in water and aqueous methanol was briefly reported previously.[36] The isolated complexes, [Cu(L-Asn)(His)]·3.5H$_2$O and [Cu(L-Asn)(His)]·0.25H$_2$O, showed approximately the same infrared (IR) spectra as those of [Cu(L-Asn)(L-His)]·4H$_2$O and [Cu(L-Asn)(L-His)], respectively. Anal. Calcd for $CuC_{10}H_{15}N_5O_5 \cdot 3.5H_2O$: C, 29.16; H, 5.38; N, 17.00. Found: C, 29.03; H, 5.33; N, 17.08. Calcd for $CuC_{10}H_{15}N_5O_5 \cdot 0.25H_2O$: C, 33.99; H, 4.42; N, 19.82. Found: C, 33.70; H, 4.42; N, 19.72.

The optical purities of histidine incorporated into the complex were determined on the basis of the CD magnitude in the d–d region and the specific rotations of histidine recovered by decomposition of the complex dissolved in water with hydrogen sulfide and subsequent column chromatography using Amberlite IR-45 in the Cl$^-$ form. The CD calibration curve was obtained by plotting the L enantiomer contents (%) of histidine against the CD magnitudes at 623 nm exhibited by the 1:1:1 Cu(II)–L-Asn–His system with different amounts of L-histidine.[35e]

B. Optical Resolution via Cu(L-Cit)(His). A solution containing DL-histidine (1.16 g, 7.5 mmol), L-citrulline (0.88 g, 5.0 mmol), and $Cu(ClO_4)_2 \cdot 6H_2O$ (1.85 g, 5.0 mmol) in 40 mL of water was neutralized (pH 7.5) with sodium hydroxide (10%). On addition of ethanol, the solution gave the complex as a blue, crystalline powder, which was collected and dried in vacuo, yield 1.5 g (70%). Anal. Calcd for $CuC_{12}H_{20}N_6O_5 \cdot 2H_2O$: C, 33.68; H, 5.65; N, 19.64. Found: C, 33.57; H, 5.51; N, 19.63.

The optical purity of histidine incorporated into the complex was determined by the CD calibration curve set up as described above.

Results

Syntheses and Infrared Spectra of Complexes. The amino acids AA with a polar group in the side chain gave the corresponding ternary copper(II) complexes Cu(His)(AA) as crystals, whereas the amino acids devoid of a polar group, such as glycine and L-valine, did not afford the ternary complexes as crystals under the present conditions. The enantiomers of asparagine showed a marked difference in the crystallization of the corresponding ternary complexes with L-histidine; so-

Table I. Stability Constants log β_{pqrs} for $Cu_p(L\text{-His})_q(AA)_r(H)_s$ in Water (25 ± 0.05 °C, I = 0.1 (KNO_3))[a]

	ternary complex[b]		binary complex[d]		protonated ligand[d]		
	Cu(L-His)(AA) (1110)		Cu(AA) (1010)	$Cu(AA)_2$ (1020)	(AA)(H) (0011)	$(AA)(H)_2$ (0012)	
AA	log β_{1110}	log β_{calcd}[c]	log β_{1010}	log β_{1020}	log β_{0011}	log β_{0012}	ref
L-Asn[e]	17.03 (0.01)	16.55	7.86	14.42	8.72	10.86	42
L-Gln	17.06 (0.02)	16.46	7.75	14.23	9.01	11.18	42
L-Ser	17.09 (0.03)	16.56	7.86	14.43	9.07	11.02	43
L-Thr	17.08 (0.02)	16.65	7.95	14.61	8.97	10.95	43
Gly	17.40 (0.02)	16.86	8.15	15.03	9.57	11.93	42
L-Ala	17.24 (0.02)	16.80	8.13	14.92	9.69	11.99	42
L-Val	17.35 (0.02)	16.80	8.05	14.91	9.57	11.83	16, 42

[a] Numbers in parentheses are estimated standard deviations. [b] This work. [c] Calculated according to eq 2. [d] These constants for the binary complexes and protonated ligands were taken from the sources cited in the right column. The following values for L-histidine taken from ref 16 were used: log β_{0101} = 9.128; log β_{0102} = 15.180; log β_{1101} = 14.089; log β_{1202} = 27.56; log β_{1201} = 23.883; log β_{1100} = 10.111; log β_{1200} = 18.078. [e] The log β_{1110} value for Cu(L-Asn)(D-His) was calculated to be 17.04 (0.01) under the same conditions.

Table II. Percentage Species Distributions in Cu(II)-Containing Ternary Systems at pH ~7 (25 °C, I = 0.1 (KNO_3))

	species distribution, %[a]		
ligand	Cu(L-His) (ligand)	$Cu(ligand)_2$	$Cu(L\text{-His})_2$
L-Asn	72	14	13
L-Gln	77	9	10
L-Ser	75	11	12
L-Thr	71	13	14
Gly	73	13	13
L-Ala	72	11	12
L-Val	73	10	11

[a] Calculated from the stability constants listed in Table I.

lutions containing equimolar amounts of Cu(II), L-His, and D-Asn usually precipitated sparingly soluble $Cu(D\text{-Asn})_2$, whose separation could be avoided by employing a Cu(II):L-His:D-Asn ratio of 1:1.3:0.9. Analogously, preparation of Cu(L-Cit)(L-His) was feasible by using more L-histidine than stoichiometrically required. For the complex with L-serine, Cu(L-His)(L-Ser), it was necessary to use twice as much L-serine because a 1:1:1 mixture gave a product that appeared to be a binary complex of L-histidine.

The ternary complexes with L-Asn, D-Asn, and L-Ser were each obtained in two modifications with different numbers of water molecules. The two forms of Cu(L-Asn)(L-His) exhibited different IR spectral patterns especially in the ranges 2300–2900 and 1600–1700 cm^{-1} probably due to different modes of hydrogen bonding in the solid state. On drying in vacuo at 60 °C, the tetrahydrate was dehydrated to give the same IR spectrum as that of the anhydrous complex, which showed a broad band in the region 2300–2900 cm^{-1} ascribable to the stretching modes of the hydrogen-bonded amide NH_2 and imidazole NH groups. This change was taken as an indication that both modifications have slightly different conformations of the side chains of coordinated ligands that are affected by water molecules of crystallization.

On the other hand, the L-homoserine-containing ternary complexes, Cu(L-His)(L-Hmser) and Cu(D-His)(L-Hmser), were obtained as trihydrates from solutions containing Cu(II), L- or D-histidine, and L-homoserine in the ratio of 1:0.9:1.3. They exhibited similar spectral patterns in the range 4000–650 cm^{-1}, whereas in the lower frequency region the patterns were appreciably different from each other. Although Cu(L-Asn)(L-His) and Cu(D-Asn)(L-His) also showed different patterns at <650 cm^{-1}, straightforward comparison may be inappropriate because of the difference in hydration in this pair of complexes. As far as histidine coordinates to copper(II) with its two nitrogen atoms in the square plane, both the active and meso forms of Cu(His)(AA) have no elements of symmetry, while the binary complexes $Cu(AA)_2$[40] and probably $Cu(His)_2$ have them to a certain degree regardless of cis–trans isomerism. This situation makes the identification of geometrical isomerism by the IR spectra more difficult.

Some diastereomers, i.e., Cu(D-His)(L-Ser), Cu(L-Gln)(L-His), and Cu(L-Cit)(D-His), were not isolated as crystals probably owing to their high solubility. Attempts to prepare the ternary complexes with aspartic acid, arginine, and ornithine, each involving a charged group in the side chain, were unsuccessful, which is in contrast with the isolation of the ternary complexes composed of Cu(II), an acidic amino acid (A), and monoprotonated arginine, ornithine, or lysine (BH).[35b]

Stability Constants. Solution equilibria of the systems involving Cu(II), His, AA, and H^+ can be expressed by the following equation using the overall stability constants β_{pqrs} (charges are omitted for simplicity):

$$pCu + qHis + rAA + sH \overset{\beta_{pqrs}}{\rightleftharpoons} Cu_p(His)_q(AA)_r(H)_s$$

$$\beta_{pqrs} = \frac{[Cu_p(His)_q(AA)_r(H)_s]}{[Cu]^p[His]^q[AA]^r[H]^s} \quad (1)$$

where Cu, His, and AA refer to unbound species and p, q, r, and s, respectively, denote the numbers of Cu(II), His, AA, and proton contained in the complex. About 40 experimental points collected for a titration of each ternary system were analyzed by the method of nonlinear least squares with the aid of the computer program SCOGS[41] using the reported values of the acid dissociation constants and the stability constants for the binary complexes.[16,42,43] After convergence of the calculation had been attained, the experimental titration curves were satisfactorily reproduced by the stability constants to within 0.02 mL (ca. 0.5%), when expressed in titer, over whole ranges of titrations. The refined stability constants are shown in Table I.

The ternary species designated as 1110 have the stability constants log β_{1110} of 17.0–17.1, some of which are slightly lower (ca. 0.4 log unit) than the reported values obtained under similar conditions.[16] Since the constants are very close to each other, they do not appear to reflect significantly the effects of the presence of polar groups in the side chains and the ligand–ligand interactions expected between them. Thus, Cu(L-His)(Gly) without the possibility of such interactions has the highest stability constant, whereas Cu(L-Asn)(L-His), for which effective interactions are expected, has the lowest. This may be due to the stability constants of the binary systems, as the higher values often gave higher stability constants of the ternary complexes. The meso system Cu(L-Asn)(D-His) has essentially the same stability constant as that of the active complex, which indicates that the stability difference that

Table III. Absorption and CD Spectral Data for Cu(II)-Containing Binary Systems at Various pH Values (I = var)

system	absorption spectra			CD spectra		
	pH	λ_{max}, nm	ϵ	pH	λ_{max}, nm	$\Delta\epsilon$
Cu(L-His)$_2$	4.9	624	51	5.1	642	0.22
	5.8	634	51	6.0	680	0.30
	6.9	644	84	6.9	680	0.38
	8.0	644	85	7.7	560	−0.03
					686	0.39
	9.6	644	87	9.7	560	−0.03
					686	0.39
Cu(L-Asn)$_2$	7.3	621	52	7.3	585	−0.05
					740	0.06
	11.2	604	55	11.2	516	−0.07
					646	0.32
Cu(L-Gln)$_2$	7.1	622	54	7.1	602	−0.11
					760	0.04
	10.5	622	55	10.5	610	−0.11
					760	0.04
Cu(L-Ser)$_2$	7.3	624	49	7.3	614	−0.18
					>780	0.02
Cu(L-Hmser)$_2$	7.1	622	49	7.1	620	−0.16
					780	0.03
Cu(L-Thr)$_2$	7.4	622	50	7.4	610	−0.21
					780	0.04
Cu(L-Ala)$_2$	7.1	622	52	7.0	625	−0.07
					>760	0.01
Cu(L-Val)$_2$	7.2	617	57	7.3	596	−0.28
					~780	0.05

arises from possible cis-trans isomerism due to the ligand-ligand interaction or other stereoisomerism is negligible under the present conditions.

We may estimate the stability constant for each ternary system according to the following equation using the stability constants for the binary species, log β_{1200} and log β_{1020}[44]:

$$\log \beta_{calcd} = \frac{1}{2}(\log \beta_{1200} + \log \beta_{1020}) + \log 2 \qquad (2)$$

where β_{calcd} denotes the estimated constant. We see from Table I that all the systems investigated have higher stability constants as compared with the calculated values.

The amounts of the species present under the experimental conditions were computed from the stability constants, and the percentage species distributions in the ternary systems at around pH 7 are listed in Table II, which reveals that the ternary species 1110 predominates at pH >6 in every system. The protonated species 1111 was negligible in solutions of pH >3.[45]

Absorption and CD Spectra. Tables III and IV present the spectral data of the binary and ternary systems at various pH values, respectively. The Cu(II)-L-His system at pH 7–10 has the absorption peak at a longer wavelength (644 nm) as compared with the peaks exhibited by the rest of the amino acids.[21] The ternary systems Cu(II)-L-His-AA almost invariably showed absorptions at around 610–620 nm at pH 7–8, whereas the systems involving iminodiacetic acid (ida) and ethylenediamine (en), Cu(II)-L-His-ida and Cu(II)-L-His-en, had peaks at 656 and 584 nm, respectively.

In neutral solution, all the ternary systems with L-AA showed positive CD extrema at 620–630 nm and those with ida and en at 640 and 580 nm, respectively. The Cu(II)-L-His system has a large positive peak at around 680 nm, while the Cu(II)-L-AA systems usually have peaks at 580–620 nm. The observed CD magnitudes with and without corrections for the species distributions at pH ~7 are presented in Table V. Owing to different ligand fields in the binary and ternary systems involving histidine, additivity of CD magnitude,[46] which has been observed to hold in the systems with an acidic and a basic amino acid (Cu(II)-L-A-L-BH),[35b] may not be allowed in these systems, but the magnitudes in the ternary systems were found to be much larger than those expected from the magnitudes exhibited by the binary systems and the distributions of the complexes in the solutions (Table V). Figure 1 shows as

Table IV. Absorption and CD Spectral Data for Cu(II)-Containing Ternary Systems at Various pH Values (I = var)

	Cu(L-His)(ligand)				
	absorption spectra			CD spectra	
ligand	pH	λ_{max}, nm	ϵ	λ_{max}, nm	$\Delta\epsilon$
L-Asn	7.3	613	57	623	0.28
	9.9	613	57	629	0.28
L-Gln	7.2	610	57	622	0.28
	10.0	610	58	624	0.29
L-Ser	4.2	665	32	636	0.06
	7.5	613	56	630	0.24
	10.3	610	58	~500	−0.01
				636	0.24
L-Hmser	7.3	610	56	620	0.26
	9.6	610	57	620	0.26
L-Thr	7.4	610	56	625	0.26
	10.2	610	57	500	−0.01
				629	0.25
Gly	4.1	670	29	635	0.07
	7.5	620	53	626	0.29
	9.9	620	54	626	0.29
L-Ala	4.1	675	21	635	0.06
	7.6	610	57	618	0.28
	9.9	610	58	618	0.29
L-Val	7.2	607	58	625	0.25
ida	4.1	720	63	610	0.02
	5.3	692	59	630	0.10
	7.0	656	57	640	0.26
	10.3	656	58	640	0.28
en	5.0	627	39	615	0.10
	7.6	584	60	580	0.20
	9.6	584	60	580	0.20

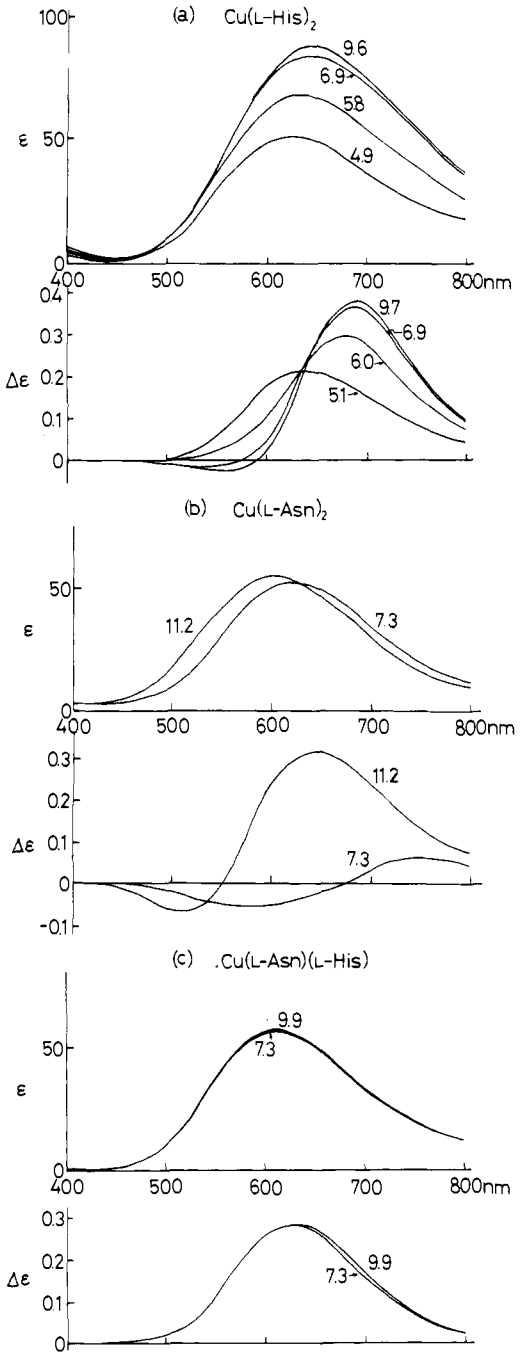


Figure 1. Absorption and CD spectra of some binary and ternary systems in the visible region. Numbers on the curves indicate the pH values of the solutions.

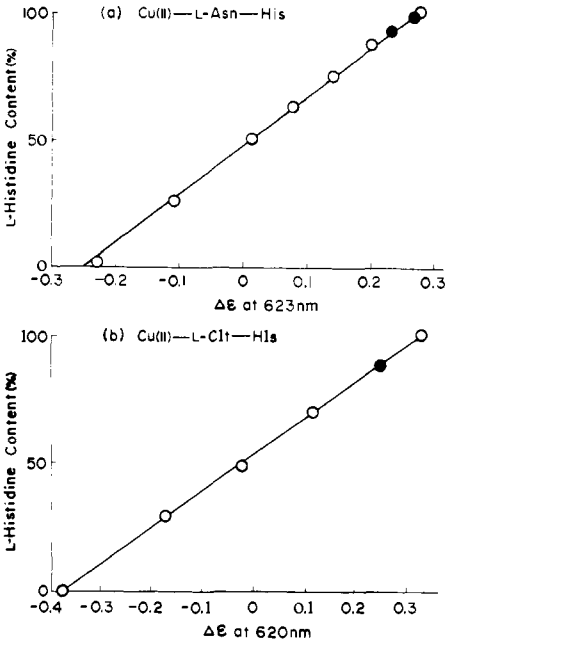


Figure 2. CD calibration curves for the systems Cu(L-Asn)(His) (a) and Cu(L-Cit)(His) (b). Open circles refer to the magnitudes exhibited by authentic samples with known optical purities and solid circles to those exhibited by the isolated complexes.

Table V. Observed and Estimated CD Magnitudes of Ternary Systems Cu(L-His)(L-AA) in the Visible Region at pH ~7 (I = 0.1 (KNO_3))

	CD magnitude ($\Delta\epsilon$) of Cu(L-His)(L-AA)			
AA	obsd	cor[a]	estd[b]	cor − estd
L-Asn	0.31	0.35	0.21	0.14
L-Gln	0.29	0.37	0.19	0.18
L-Ser	0.25	0.34	0.11	0.23
L-Thr	0.27	0.39	0.13	0.26
Gly	0.29	0.37	0.23	0.14
L-Ala	0.29	0.40	0.18	0.22
L-Val	0.26	0.34	0.07	0.27

[a] Corrected for the species distribution (Table II) by using the values for $Cu(L\text{-}AA)_2$ and $Cu(L\text{-}His)_2$, $\Delta\epsilon_{Cu(L\text{-}AA)_2}$ and $\Delta\epsilon_{Cu(L\text{-}His)_2}$, respectively. [b] Estimated values ($\Delta\epsilon_{calcd}$) were obtained by the equation $\Delta\epsilon_{calcd} = {}^1/_2(\Delta\epsilon_{Cu(L\text{-}AA)_2} + \Delta\epsilon_{Cu(L\text{-}His)_2})$.

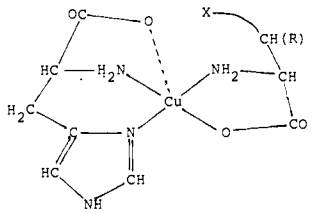
an example the absorption and CD spectra exhibited by the systems $Cu(L\text{-}His)_2$, $Cu(L\text{-}Asn)_2$, and Cu(L-Asn)(L-His) in neutral to alkaline solution.

Optical Resolution. Assuming the solubility difference as seen in the synthesis of the diastereomeric complexes such as Cu(L-Asn)(L-His) and Cu(D-Asn)(L-His), we attempted the optical resolution of DL-histidine via formation of the ternary complexes with L-asparagine and L-citrulline, both of which exhibited the most striking difference in crystallization. Selective incorporation of an enantiomer of histidine was determined for these complexes isolated in the analytically pure state, according to the CD calibration curves shown in Figure 2.[35c] The results are summarized in Table VI. The L-enantiomer content of histidine incorporated into one of the two modifications of the complex with L-asparagine was found to be as high as 98%, and it was further confirmed by the specific rotation of histidine separated from the complex.[36] The ternary complex with L-citrulline also incorporated the L-enantiomer of histidine preferentially (ca. 78%) from a solution containing $Cu(ClO_4)_2$, L-citrulline, and DL-histidine in the molar ratio of 1:1:1.5. Such stereoselectivity was not observed for the ternary systems with L-glutamine, L-threonine, and L-serine, either because isolation of analytically pure complexes was unsuccessful or because the complexes equally incorporated the L and D enantiomers of histidine under the conditions employed.

Discussion

Preferential Formation of Cu(His)(AA). 1. Synthetic and Spectroscopic Studies. Until recently, Cu(L-His)(L-Thr) has been the only histidine-containing ternary amino acid–copper(II) complex isolated and crystallographically analyzed. The present synthetic study shows that all combinations of histidine and an amino acid having a polar side chain (AA)

around copper(II) afford the ternary complexes Cu(His)(AA) as crystals. Assuming that Cu(L-His)(AA) have the same mode of coordination as has been clarified for Cu(L-His)(L-Thr),[8] we see that the polar side group (X) of AA can be in the vicinity of the oxygen of the apically coordinated carboxyl group (structure **1**), and it is not improbable that both groups



Cu(L-His)(L-AA)

1

AA	R	X
Asn	H	$CONH_2$
Gln	H	CH_2CONH_2
Ser	H	OH
Hmser	H	CH_2OH
Thr	CH_3	OH
Cit	H	$CH_2CH_2NHCONH_2$

interact with each other through a hydrogen bond under favorable conditions. Isolation of a complex does not simply implicate its preferred formation but is affected by solubility and presence of polar groups that assist the crystal growth through intermolecular noncovalent bondings. In this respect, the polar side groups of amino acids AA are supposed to contribute to such bondings and favor crystallization. However, the solubility alone does not explain the synthesis of Cu(His)(AA), because some binary complexes, such as $Cu(Asn)_2$, $Cu(Gln)_2$, and $Cu(Cit)_2$, are much less soluble than the corresponding ternary complexes, and the ternary systems containing Cu(II), L-histidine, and glycine or L-valine in place of AA at various ratios indeed gave the binary complexes of these amino acids. It was briefly referred to in the Results section that combination around copper(II) of aspartic or glutamic acid (A) with a monoprotonated basic amino acid (BH) has led to formation and isolation of the corresponding ternary complex Cu(A)(BH) involving both amino acids.[35b] This complex formation was found to be accompanied by an unusual CD magnitude enhancement in the d-d region, which was attributed to increased rigidity as a result of intramolecular electrostatic bonds between the oppositely charged side groups.[35a,b] Although the ligand fields in $Cu(His)_2$, $Cu(AA)_2$, and Cu(His)(AA) are different, the CD extrema being compared for estimation of the CD magnitudes for the ternary systems are probably associated with the same electronic transitions, $a_{xy} \rightarrow d_{x^2-y^2}$ and $d_{yz} \rightarrow d_{x^2-y^2}$, and we may therefore estimate the magnitudes by the mean values of the magnitudes for $Cu(His)_2$ and $Cu(AA)_2$ at the maximum wavelength. The observed magnitudes for the Cu(L-His)(L-AA) systems are larger than the estimated values (Table V). The anomalous magnitudes may have resulted from the combination of the imidazole ring as a π acceptor and the carboxylate oxygen as a π donor in the copper(II) coordination plane. As has been reported for the ternary systems involving coordinated aromatic nitrogens,[35c,47] the presence of a π acceptor and a π donor affects the d-d transitions, and this is the case with the present systems. Their CD spectral magnitudes implicate that both L-histidine and an amino acid are bound to copper(II) at pH >7, although we are unable to evaluate the magnitude enhancement specifically due to the association of the side groups in these systems. The absorption maxima at 610-620 nm observed for the Cu(II)-L-His-L-AA systems show that, irrespective of the polar or charged groups present in the side chains of AA, the ternary complexes have the same set of donor groups in the square-planar coordination sites of copper(II). All these observations may suggest the existence of the molecular arrangement that assists the intramolecular hydrogen bonding as a weak but significant driving force for ternary complex formation.

2. Solution Equilibria. The stability constants, log β_{1110}, for all the ternary complexes, Cu(L-His)(L-AA), fall in the range 17.03-17.09, whereas the values for Cu(L-His)(Gly), Cu(L-His)(L-Ala), and Cu(L-His)(L-Val) are slightly higher (Table I). The systems investigated exhibit higher stability constants than those estimated by eq 2, suggesting the stabilization due to the histidine imidazole as a π acceptor. However, it is difficult to assess the possible effects of ligand-ligand interactions on the complex stability from these constants. Since hydrogen bondings that are expected between the side groups should be seriously affected by the ionic strength of solution and the polarity of the solvent used, their effects may not be explicitly reflected in the stability constants determined in water at I = 0.1. In addition, the strain due to the ligand-ligand interactions may decrease the stability by affecting the ΔH value (vide infra).

The mode of coordination by asparagine in solution has been discussed by Baxter and Williams,[15] who determined the thermodynamic parameters for the formation of $Cu(L\text{-}Asn)^+$, $Cu(L\text{-}His)^+$, and Cu(L-Asn)(L-His) by calorimetric measurements in water at I = 3.0 ($NaClO_4$): the ΔH and ΔS values for $Cu(Asn)^+$ suggested either that the amide group is weakly localized near the axial bond of copper(II) or that there is a mixture of bi- and terdentate asparagine complexes. Our view that there is an interaction between the carboxylate oxygen of L-histidine and this amide group is in line with their result. The rather low value of $-\Delta H$ observed for Cu(L-Asn)(L-His) was inferred to result from either the liberation of water molecules from solvated copper(II) or increased bond strain caused by ternary complex formation.[15] It might be interpreted as partly due to the strain imposed by the intramolecular bond in spite of the high ionic strength. On the other hand, the weak apical interaction of the hydroxyl group of serine has been inferred by Pettit and Swash[43] on the basis of the ΔH values supporting the preferential formation of $Cu(L\text{-}Ser)_2$ as compared with Cu(D-Ser)(L-Ser), although the oxygen-copper(II) interaction would be mediated by a water molecule coordinated to copper(II) because of the short side chain length of serine. As supported by the X-ray analyses[8,9] as well as the calorimetric,[12,15] potentiometric,[7,48] and spectral[21,23] studies on $Cu(L\text{-}His)_2$ and the ternary complexes, an apical position in Cu(L-His)(AA) in solution is probably occupied by the L-histidine carboxyl oxygen, so that all the above observations are compatible with the intramolecular hydrogen bond between this oxygen and the polar side group of AA.

3. Optical Resolution. Under favorable conditions, stereoselectivity in ternary complex formation leads to optical resolution of ligands such as α-amino acids.[35d,e] Because the diastereomeric systems Cu(L-Asn)(L-His) and Cu(L-Asn)(D-His) exhibited the same stability constants in water at I = 0.1 (Table I), the preferential incorporation of the L enantiomer of histidine into the ternary complex with L-asparagine may be mainly governed by the solubility factor under the experimental conditions, and the same may be true for the L-Cit-containing system. Freeman and Martin[11] also reported the absence of stereoselectivity in the formation of Cu(His)(Thr) at I = 0.1 (KNO_3). However, Table VI indicates that Cu(L-Asn)(His) isolated from aqueous methanol (or ethanol) solution incorporated L-histidine more selectively than that obtained from aqueous solution. On the solubility basis alone, addition of methanol or ethanol should lower the solubilities of both diastereomers, Cu(L-Asn)(L-His) and Cu(L-Asn)(D-His), and thus result in less selective incorporation of an enantiomer. The present observation can be interpreted in terms of the strengthened intramolecular hydrogen bonding in a less polar solvent, which probably increases the ligand selectivity around copper(II). The difference in crystallization may be ascribed either to cis-trans isomerism such as proposed for Cu(L-A)(L-BH) and Cu(D-A)(L-BH) with electrostatic ligand-ligand interactions,[35b] or to the absence of the intra-

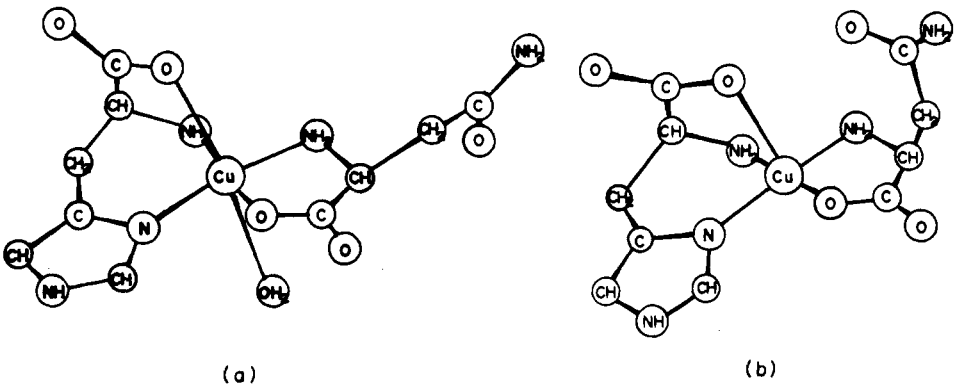
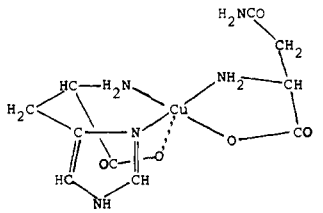


Figure 3. Molecular structures of (a) [Cu(L-Asn)(L-His)(H_2O)]·3H_2O and (b) [Cu(L-Asn)(L-His)].[49] The rest of the water molecules in (a) occupy the space between the complex molecules and are hydrogen bonded to neighboring polar groups, such as the amide and the α-amino group of L-asparagine and the carboxylate group of L-histidine, forming the structural network of the crystal.

molecular hydrogen bond in Cu(L-Asn)(D-His) assuming a cis arrangement in the copper(II) coordination plane (structure **2**). In this connection, Brookes and Pettit[16,48] detected slight



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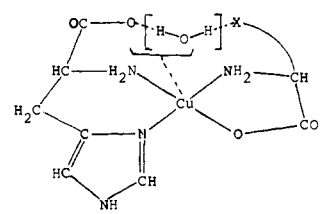
but significant stereoselectivity in the formation of ternary histidine–copper(II) complexes with the L enantiomers of monoprotonated basic amino acids (L-B'H) and concluded that the preferential formation of Cu(L-His)(L-B'H) over Cu(D-His)(L-B'H) was due to the electrostatic bond present in the former between the histidine carboxyl oxygen and the protonated side group of B'H with a cis configuration presupposed. This explanation may also be applied to the present cases.

Structures of Ternary Complexes. As is evident from the foregoing discussion, the observations in support of the intramolecular hydrogen bond lead to the structure for Cu(L-His)(L-AA), which involves a terdentate histidine and a bidentate AA coordinated around copper(II) in a cis configuration with an intramolecular hydrogen bond with or without intervening water (structure **3**). This configuration of donor



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atoms in the square plane is the same as that disclosed by X-ray analysis of Cu(L-His)(L-Thr).[8] Very recently X-ray analyses have been performed on the two modifications of the ternary complex with L-asparagine, [Cu(L-Asn)(L-His)] and [Cu(L-Asn)(L-His)]·4H_2O, both of which have been shown to possess analogous structures to the structure of Cu(L-His)-(L-Thr) involving no intramolecular ligand–ligand interactions (Figure 3).[49] Interestingly, however, the two structures exhibit different conformations of the side chain of L-asparagine: a stretched side chain for the tetrahydrated complex and a bent side chain for the anhydrous complex, with both amide groups hydrogen bonded to adjacent complex molecules and/or water molecules. Since such a conformational difference obviously originates from the solvents used for crystallization, we may infer that the crystal growth has been accomplished by intermolecular hydrogen bonds at the expense of the hydrogen bond between the amide NH_2 and the apical oxygen favored in nonaqueous solution, resulting in the bent form of the side chain in the solid state. The X-ray structural data disclosed that the distance between the carboxyl oxygen and the amide oxygen is 0.354 nm in the anhydrous complex. Therefore, the amide nitrogen may be able to have access to the carboxyl oxygen to within the hydrogen bond length by rotation around the C_β–C_{amide} bond and a slight deformation of the chelate ring, which further supports the above view (Figure 4). In the binary complex, [Cu(L-Asn)$_2$], in the solid state, L-asparagine is coordinated in a trans configuration and its amide group is stretched to be apically coordinated to the adjacent copper-(II).[50]

Although no X-ray analysis has been carried out for the other ternary complexes synthesized in this study, their spectral similarities suggest the same arrangement in the coordination plane as that for Cu(L-Asn)(L-His) and the conformational difference observed for Cu(L-Asn)(L-His) might also be possible for those complexes under certain conditions. Considering that the histidine-containing ternary amino acid–copper(II) complexes usually assume a cis configuration in the solid state,[51] the active complexes, Cu(L-His)(L-AA), must retain the possibility of the direct or water-mediated intramolecular hydrogen bond at least in less polar solvents.[52] That the carboxylate oxygens in Cu(L-His)(D-His) in the solid state[9] have been found to be bridged to the apically coordinated water molecules through hydrogen bonding is highly significant in the sense that in the ternary complexes either the carboxylate group or a polar group might take the place of the water molecules. Based on the X-ray study, Camerman et al.[9] proposed an analogous structure for Cu(L-His)$_2$ in solution with one of the carboxylate group hydrogen bonded in the same way. From these considerations, we are disposed to infer that the structure of Cu(L-His)(L-Thr), which does not have the hydroxy group of L-threonine in the propinquity of the carboxyl oxygen of L-histidine, represents one of the possible conformational isomers. Its disclosed structure in the solid state[8] can be reconciled with the proposed structure in solution involving an intramolecular bond by the rotation around the C_α–C_β bond of L-threonine, which is shown by space-filling models to place the hydroxyl group at around the hydrogen bond distance from the carboxyl oxygen.

As regards the meso complexes, Cu(D-His)(L-AA) or Cu(L-His)(D-AA), both cis and trans forms are possible on the basis of steric requirements. Although the cis configuration

Table VI. Optical Resolution of DL-Histidine via Formation of Cu(L-Asn)(His) and Cu(L-Cit)(His)

system	complex isolated	yield, %[a]	optical purity of incorporated L-histidine, %	
			CD calibration curve	specific rotation
Cu(II)-L-Asn-DL-His[b] (in aqueous methanol)	$[Cu(L\text{-}Asn)(His)]\cdot 0.25H_2O$	25	98	98
Cu(II)-L-Asn-DL-His[b] (in water)	$[Cu(L\text{-}Asn)(His)]\cdot 3.5H_2O$	24	84	88
Cu(II)-L-Cit-DL-His (in aqueous ethanol)	$[Cu(L\text{-}Cit)(His)]\cdot 2H_2O$	70	78	*c*

[a] The yield of the isolated complex is based on the amount of copper(II) used. [b] Taken from ref 36. [c] Not measured.

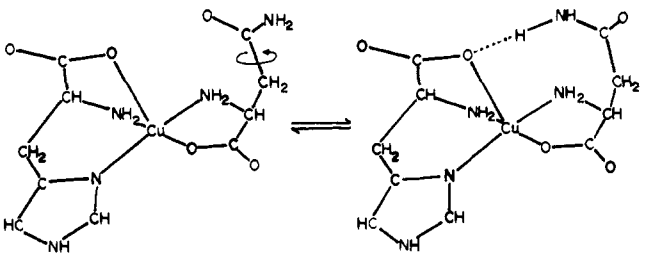

Figure 4. Possible side-chain conformations in Cu(L-Asn)(L-His).

of the ternary histidine complexes appears to be favored owing to the repulsion between the hydrogens of the α-amino group of AA and imidazole C-2 of histidine, the trans configuration revealed in Cu(L-His)(D-His) indicates that even the less favorable trans form is preferred in the presence of a more serious steric hindrance arising from two imidazoles in a cis arrangement. Therefore, the geometry in the square plane seems to depend considerably on the degree of steric crowdedness around the coordinating nitrogen atoms, and, under circumstances where intramolecular interactions are important, the meso complexes could have a trans configuration, which would make such interactions feasible.

Possible Biological Significance. Biological systems contain a vast number of potential low-molecular-weight ligands, and information from simplified systems must be of importance for understanding the behaviors of transition-metal ions in living organisms. The present observations suggest that under favorable conditions inter- and intramolecular noncovalent bonds may have considerable influence on the properties of complexes. Such bonds may increase stereoselectivity or ligand selectivity around the central metal ion in a ternary complex by the steric requirements for the best fit of the interacting groups from both ligands. Specifically, the formation of Cu(L-Asn)(L-His), Cu(L-Gln)(L-His), and Cu(L-His)(L-Thr), which have been reported to be predominant low-molecular-weight copper(II) complexes in human blood serum, can be explained in terms of intramolecular hydrogen bonds: such species would be particularly favored in the transport of copper(II) in hydrophobic environments such as biological membranes. The apparent inconsistency of the conclusions on the species distribution in human blood serum, reached from the tracer studies[26,28] and from the computer simulations,[31-33] is inferred to arise partly from the difference in the effects of ionic strength in biological systems containing macromolecules and in model systems with low-molecular-weight ligands only. However, the preference of formation of the above ternary complexes should be carefully estimated, because the ligand-ligand interaction as a driving force for ligand selectivity is by nature seriously affected by various ions present, and concentrations of ligands in blood are certainly another factor to be taken into consideration.

Acknowledgments. We thank Mr. Tsuneji Ueki and Mr. Masahiro Yamamoto for technical assistance and Miss Teruko Kuroe for elemental analysis. We are also indebted to Professor Eishin Kyuno and Dr. Shigeo Nakagawa of Hokuriku University for thermal analysis. The computation of the stability constants was performed at the Osaka University Computer Center, to which our thanks are due. This work was supported in part by a grant from the Ministry of Education of Japan.

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(51) According to the personal communication from Professor Y. Sasada, newly prepared Cu(L-Ala)(L-His)·$4H_2O$ was disclosed by X-ray analysis to have a cis arrangement with the histidine carboxyl group hydrogen bonded to the apically coordinated water molecule.
(52) Recent NMR studies on the corresponding ternary complexes of palladium(II) provide evidence in support of the intramolecular ligand-ligand interactions (O. Yamauchi and A. Odani, results partly presented at the ACS/CSJ Chemical Congress, Honolulu, April 1979). From the comparison of the spectral patterns of the systems, such as Pd(L- or D-His)(L-AA) (AA = Hmser or Gln), Pd(Ha)(L-AA) (Ha = histamine), Pd(L-Ala)(L-AA), and Pd(L-AA)$_2$, the spectral patterns of coordinated L-AA have been found to be affected by the presence of the carboxylate group of histidine. The result indicates the conformational change of L-AA due to the interaction with the carboxylate group, and further points to similar interactions in the copper(II) complexes, since both Cu(II) and Pd(II) prefer a square-planar structure and the apical bonding present in Cu(II) but probably absent in Pd(II) is not supposed to interrupt the intramolecular ligand–ligand interaction.

Structure of a 12-Vertex Arachno Carborane, σ-(η^5-C_5H_5)Co(η^5-C_5H_4)$^+$—$(CH_3)_4C_4B_8H_8^-$, an Analogue of $B_{12}H_{12}^{6-}$ and $C_2B_{10}H_{12}^{4-}$. Mechanisms of Carborane Fluxional Behavior and Metallocarborane Formation from the $(CH_3)_4C_4B_8H_8^{2-}$ Ion[1]

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Abstract: The structure of the title compound, the first confirmed example of a 12-vertex arachno borane cage, was determined by single-crystal X-ray diffraction and is formulated as a cobaltocenium-substituted derivative of the $(CH_3)_4C_4B_8H_9^-$ ion, which in turn is obtained by protonation of the $(CH_3)_4C_4B_8H_8^{2-}$ dianion. The carborane polyhedron, a 30-electron, 12-vertex cage, has an open basket-like geometry in which the four C-CH_3 groups occupy contiguous positions on the open face. One of the C-CH_3 units is coordinated to only two framework atoms, and the "extra" hydrogen atom is attached to this bridging carbon. The methyl group on the bridging carbon atom is located over the open face of the cage, and the cobaltocenium substituent is attached to a boron atom on the open face adjacent to the bridging carbon. From this structure and the previously determined geometry of neutral $(CH_3)_4C_4B_8H_8$, a mechanism is proposed for the formation of the dianion and for its fluxional behavior in solution. Based on this mechanism, a scheme is proposed to account for the formation of several tetracarbon ferracarboranes from the $(CH_3)_4C_4B_8H_8^{2-}$ dianion. The interconversion of neutral $(CH_3)_4C_4B_8H_8$ isomers and the probable structure and fluxionality of isomer B are discussed. The σ-$(C_5H_5)Co(C_5H_4)^+$-$(CH_3)_4C_4B_8H_8^-$ molecule crystallizes in the triclinic $I\bar{1}$ space group with $a = 8.047$ (3) Å, $b = 37.05$ (2) Å, $c = 21.551$ (9) Å, $\alpha = 90.04$ (6)°, $\beta = 97.13$ (8)°, $\gamma = 89.94$ (7)°, and $V = 6375$ (3) Å^3; $R = 0.047$ for the 7516 reflections for which $F_o^2 > 3\,\sigma(F_o^2)$. There are 12 molecules in the unit cell and three molecules per asymmetric unit. Full-matrix least-squares refinement of the positional and thermal parameters of all 168 atoms (including all hydrogens) in the asymmetric unit gave a final R value of 0.047.

The stability of the icosahedron as a structural unit in boron chemistry is well known.[2] Molecular orbital calculations[3,4] on the $B_{12}H_{12}^{2-}$ ion indicate the presence of 13 bonding molecular orbitals, which is precisely the number of bonding electron pairs that are available for linking the 12 BH units in that species. The icosahedral carborane isomers, 1,2-, 1,7-, and 1,12-$C_2B_{10}H_{12}$, are isoelectronic with $B_{12}H_{12}^{2-}$ and similarly contain 26 skeletal electrons in 13 bonding MOs exclusive of C–H and B–H bonding.[2]

If an electron pair is added to any of these filled-shell icosahedral systems, forming 28-electron $B_{12}H_{12}^{4-}$, $C_2B_{10}H_{12}^{2-}$, or $C_4B_8H_{12}$ species, one expects distortion of the polyhedral framework; however, it is now clear that the nature of this distortion can, and does, vary widely in different systems. Although the prototype borane $B_{12}H_{12}^{4-}$ is unknown and the structures of the $C_2B_{10}H_{12}^{2-}$ isomers have not been established, X-ray diffraction studies of the 28-electron[5] species $R_2C_2B_{10}H_{11}^-$ (R = CH_3[6] or C_6H_5[7]), $(CH_3)_4C_4B_8H_8$,[8] (η^5-

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