MIXED α-AMINO ACID-COPPER(II) COMPLEXES WITH LIGAND-LIGAND INTERACTIONS

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Chapter 1

Introduction

It has been firmly established that transition metals, such as Mn, Fe, Co, Cu, Zn and Mo play vital roles in a vast range of biological processes, particularly in the reactions catalyzed by metalloenzymes.[1] Approaches not only from biology but also from chemistry with appropriate low molecular weight model complexes have contributed much to the understanding of the biological processes with metal ion participation.

Biological systems make use of a variety of suitable non-covalent interactions, i. e. electrostatic interaction, hydrogen bonding, hydrophobic interaction and charge transfer interaction, as a source of high specificity, reaction acceleration and the like.[2] For instance, the guanidinium group of arginine 145 residue in a zinc(II)-containing enzyme, carboxypeptidase A, electrostatically interacts with the terminal caboxylate group of a substrate peptide to fix its susceptible bond at a suitable position for the enzyme's exopeptidase activity(Figure 1).[3] Trypsin, an endopeptidase, also binds a substrate at its aspartic acid 189 residue through the electrostatic interaction with the positively charged side chain of an internal basic amino acid residue of the substrate.[4]

In spite of the recognition of their importance as potential biological models, few mixed ligand complexes have been specifically designed as models for biological processes with metal ion participation. This study is intended to shed light on the diverse and profound roles of noncovalent interactions in biological processes through the properties of novel types of mixed ligand copper(II) complexes of α-amino acids with intramolecular ligand-ligand interactions(Scheme 1).

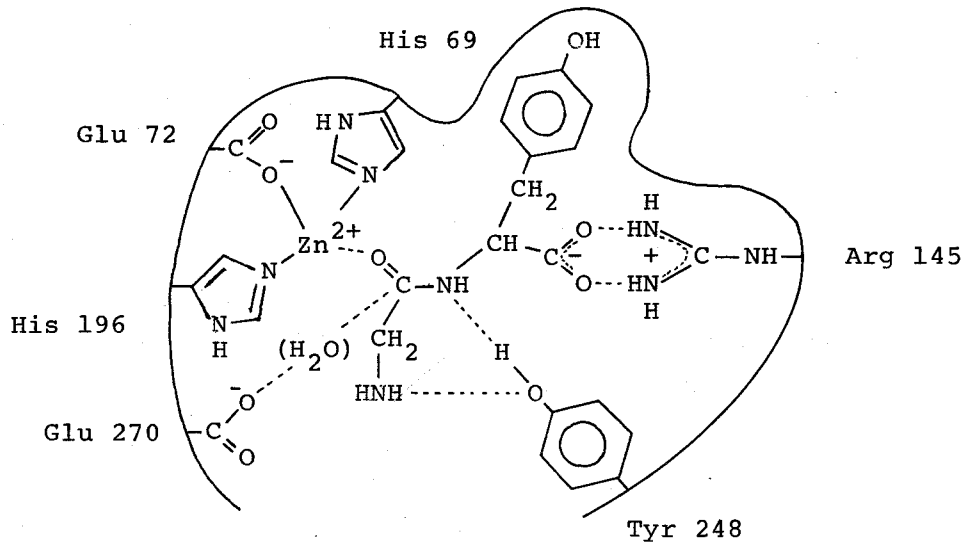
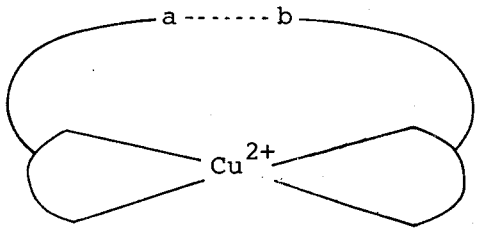


Figure 1. A Schematic Representation of the Carboxypeptidase A —glycyl-L-tyrosine Complex(Taken from Reference 3).



Scheme 1. A Model Ternary Copper(II) Complex with Intramolecular Ligand-Ligand Interaction.

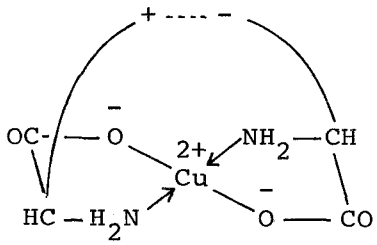
Chapter 2

Preparation and Spectral Properties of Mixed α-Amino Acid-Copper(II) Complexes with Intramolecular Electrostatic Ligand-Ligand Interactions

Biological compounds are provided with functional groups, many of which carry a positive or negative charge under physiological conditions. When oppositely charged groups approach each other within a certain distance, they form electrostatic bonds to make essential contribution in a variety of enzymatic and other biological processes. In proteins, such an interaction occurs between a negatively charged side chain of aspartic or glutamic acid and a positively charged side chain of arginine, lysine or histidine. Terminal carboxylate and protonated amino groups of proteins also form ionic bondings at pH 7-8.

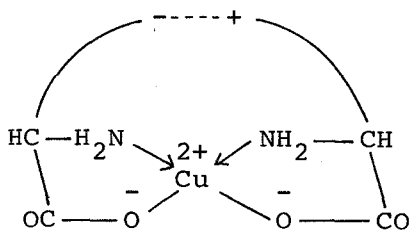
A mixed ligand metal complex with intramolecular electrostatic ligand-ligand interactions was designed as a simplified model for the enzyme-substrate complex formed in the course of the metalloenzyme-catalyzed reaction. For the present purpose, L- and D-aspartic acid(Asp; also abbreviated as $aspH_2$) and L- and D-glutamic acid(Glu; $gluH_2$) were employed as ligands with a negatively charged side chain(referred to as AH_2 hereinafter) and L-arginine(Arg, $argH_2$), L-lysine(Lys; $lysH_2$) and

L-ornithine(Orn; $ornH_2$) as ligands with a positively charged side chain(referred to as BH_2). The space-filling models suggested a *trans* structure 1 for the active form complex [Cu^{II}-(L-A)(L-BH)] and a *cis* structure 2 for the *meso* form complex [Cu^{II}(D-A)(L-BH)] because of the steric requirements imposed by the electrostatic ligand-ligand interactions. In order to get information on this point, the isolation and characterization of the complex for all combinations of AH_2 and BH_2 were attempted.



[Cu^{II}(L-A)(L-BH)]

1



[Cu^{II}(D-A)(L-BH)]

2

A : asp, glu

B : arg, lys, orn

Experimental

Materials

D-Arginine hydrochloride and D-lysine hydrochloride were obtained from Fluka AG and D-ornithine hydrochloride from Sigma Chemical Co. All other amino acids were purchased from Nakarai Chemicals Ltd. They were used without further purification. All other materials used were of reagent grade or of highest grade available.

Preparation of Mixed Ligand Copper(II) Complexes

The complexes were prepared by essentially the same procedure as typically described below for [Cu(L-glu)(L-argH)]. Copper(II) perchlorate hexahydrate(1.85 g, 5 mmol), L-arginine hydrochloride(1.05 g, 5 mmol) and L-glutamic acid (0.74 g, 5 mmol) were dissolved in *ca.* 30 ml of water, and the pH of the resulting solution was adjusted to *ca.*9 with aqueous sodium hydroxide. The reaction mixture was stirred for 1 h at room temperature and concentrated *in vacuo* to a small volume at temperatures below 50°C. Addition of methanol to the residue gave blue crystals, which were recrystallized from aqueous methanol-ethanol.

The pH of the reaction mixture containing L-ornithine or L-lysine as BH_2 ligands was adjusted to *ca.* 7.

The analytical data for the isolated complexes are shown in Table 1.

Measurements

Infrared spectra were obtained in the range 4000-650 cm^{-1} with a Hitachi EPS-S2 or a Hitachi 215 grating infrared spectrophotometer with the KBr disk method and in the range 700-200 cm^{-1} with a Hitachi EPI-L grating infrared spectrophotometer in the dry air with the Nujol mull method.

The spectroscopic measurements were performed in water or in aqueous ethanol at room temperature for the systems with the copper(II) : AH_2 : BH_2 ratios of 1 : 1 : 1(ternary systems) and 1 : 2 : 0 and 1 : 0 : 2(binary systems) at pH 5-11 at a constant copper(II) concentration of $5x10^{-3}$ M. The spectral samples were prepared from 0.1 M stock solutions of the ligands and copper(II) perchlorate. The pH values were roughly adjusted with aqueous sodium hydroxide and dilute perchloric acid and finally determined after the spectroscopic measurements. For most spectral measurements ionic strengths were not adjusted at a constant value(I=variable) to avoid weakening of the expected ligand-ligand interactions by higher ion concentrations. Absorption spectra were recorded in the range 400-800 nm on a Union Giken SM-401 High Sensitivity recording spectrophotometer. Circular dichroism(CD) spectra were measured in the range 350-800 nm with a JASCO MOE-1 spectropolarimeter.

Results and Discussion

Preparation of Mixed Ligand Copper(II) Complexes

As shown in Table 1, all the expected ternary complexes except [Cu(L-asp)(L-ornH)] were isolated as crystals in good yields. Although it was difficult to obtain pure crystals of [Cu(L-asp)(L-ornH)], its formation is assumed from analytical data.[5]

The structures of these complexes are expected from the space-filling models to be such as are expressed by 1 and 2, which suggest that the L-AH_2-L-BH_2 pairs of ligands favor the *trans* structure 1 around the central copper(II) ion, whereas the D-AH_2-L-BH_2 pairs prefer the *cis* structure 2. Both structures are more puckered and may be sterically less stable as compared with those without ligand-ligand interactions, but the synthetic results seem to indicate the existence of the electrostatic bondings, which supplement the steric disadvantage incurred. Although the complexes with pairs of AH_2 and BH_2 containing longer side chains, e.g. [Cu(glu)(argH)] and [Cu(glu)(lysH)] were isolated easily, the total length of the two side chains may not be the sole factor affecting the isolation, because [Cu(L-glu)(L-ornH)] was more easily isolated than [Cu(L-asp)(L-lysH)], which has the same side chain length as the former. The difficulty encountered in isolation of [Cu(asp)(L-ornH)] with the shortest chain length nevertheless suggests that the chain length as well as the solubility

Table 1. Elemental Analysis of Mixed Ligand Copper(II) Complexes of α-Amino Acids

Complex	Formula	Found(%)			Calcd(%)		
		C	H	N	C	H	N
[Cu(L-glu)(L-argH)]·$2H_2O$	$C_{11}H_{21}N_5O_6Cu \cdot 2H_2O$	31.45	5.64	16.77	31.54	6.02	16.72
[Cu(D-glu)(L-argH)]·H_2O	$C_{11}H_{21}N_5O_6Cu \cdot H_2O$	32.71	5.66	17.44	32.96	5.78	17.47
[Cu(L-asp)(L-argH)]·H_2O	$C_{10}H_{19}N_5O_6Cu \cdot H_2O$	30.77	5.59	18.17	31.05	5.47	18.11
[Cu(D-asp)(L-argH)]·$1.5H_2O$	$C_{10}H_{19}N_5O_6Cu \cdot 1.5H_2O$	30.10	5.47	17.49	30.34	5.60	17.69
[Cu(L-glu)(L-lysH)]·$1.5H_2O$	$C_{11}H_{21}N_3O_6Cu \cdot 1.5H_2O$	34.52	5.97	11.19	34.59	6.34	11.01
[Cu(D-glu)(L-lysH)]·H_2O	$C_{11}H_{21}N_3O_6Cu \cdot H_2O$	35.49	5.81	11.29	35.43	6.22	11.27
[Cu(L-asp)(L-lysH)]·$1.5H_2O$	$C_{10}H_{19}N_3O_6Cu \cdot 1.5H_2O$	32.89	5.84	11.41	32.65	6.02	11.42
[Cu(D-asp)(L-lysH)]·H_2O	$C_{10}H_{19}N_3O_6Cu \cdot H_2O$	33.51	5.79	11.90	33.66	5.90	11.71
[Cu(L-glu)(L-ornH)]·$2H_2O$	$C_{10}H_{19}N_3O_6Cu \cdot 2H_2O$	32.05	5.63	11.28	31.87	6.14	11.15
[Cu(D-glu)(L-ornH)]·$2H_2O$	$C_{10}H_{19}N_3O_6Cu \cdot 2H_2O$	31.85	5.71	11.45	31.87	6.14	11.15
[Cu(D-asp)(L-ornH)]·$2.5H_2O$	$C_9H_{17}N_3O_6Cu \cdot 2.5H_2O$	28.92	5.39	11.34	29.07	5.97	11.30

and *cis-trans* geometry around the copper(II) ion exerts influence over the formation and crystallization of the complexes.

IR Spectra and *cis-trans* Isomerism

Figure 2 shows the IR spectra of the mixed ligand complexes in the range 4000-650 cm^{-1}. The spectral patterns in the fingerprint region are different from those of the corresponding binary complexes. Subtle spectral differences in this region are also detected between the Cu(L-A)(L-BH) and Cu(D-A)(L-BH) series with all combinations of AH_2 and BH_2 except the Asp-Orn pair, which may reflect the structural differences as illustrated by 1 and 2.

Geometrical isomerism has been known to give rise to characteristic bands due to metal-ligand bonds in the range below 700 cm^{-1}.[6] Thus, the spectral patterns of *cis-* and *trans-*Cu(II) glycinate in this region[7] are considerably different from each other. It is seen from Figure 3 that at around 500 cm^{-1} and 350-200 cm^{-1} the complexes with L-AH_2-L-BH_2 pairs give the spectra that differ from those exhibited by the complexes [Cu(D-A)(L-BH)]. This suggests the presence of the *cis-trans* isomers expressed by 1 and 2, and since the side chains in these systems are comparable, the isomerism in turn points to the intramolecular ligand-ligand bondings.

Absorption and Circular Dichroism Spectra

The CD spectra obtained for each of the systems at var-

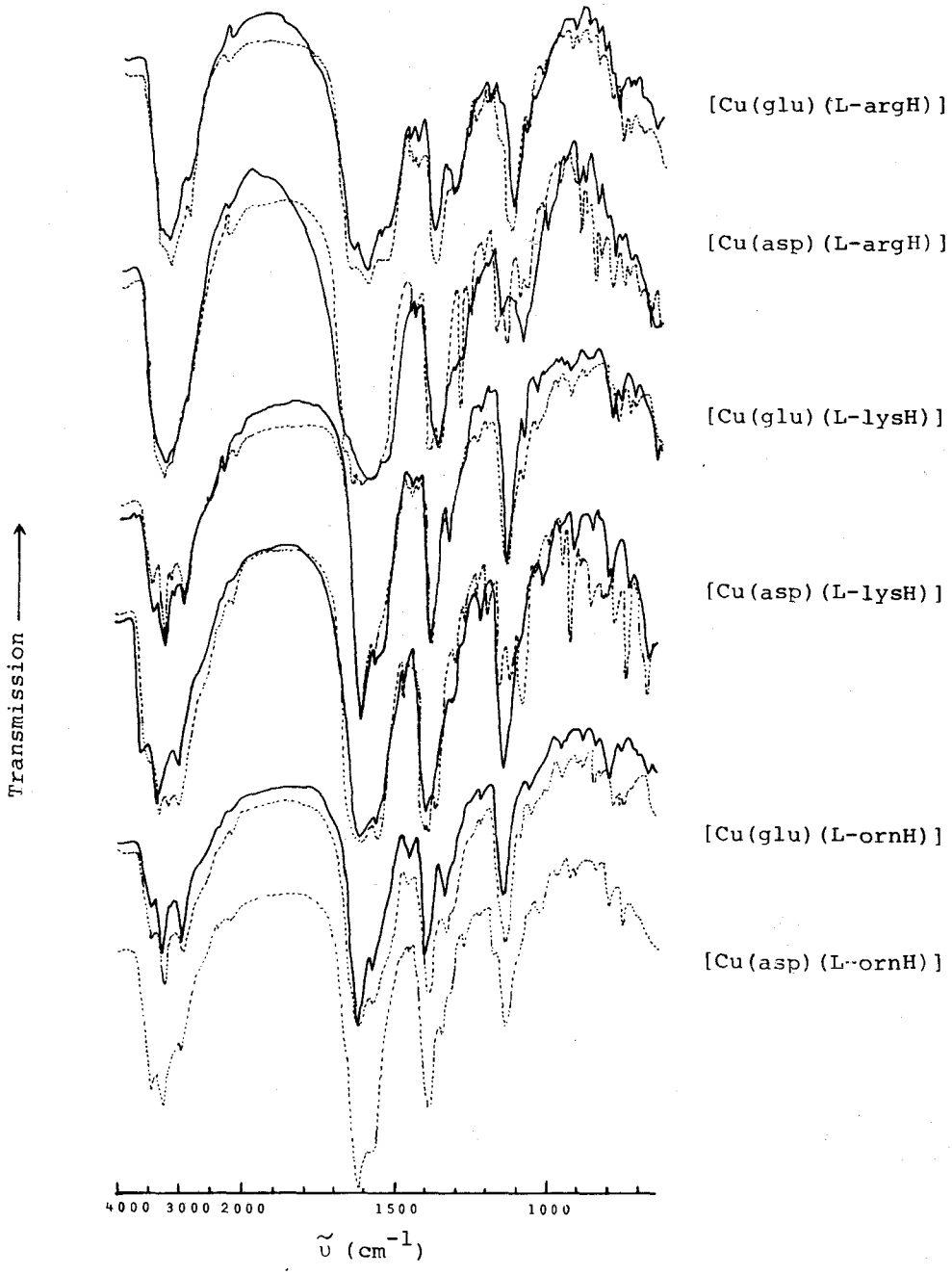


Figure 2. Infrared Spectra of Copper(II) Complexes in the Range 4000-650 cm^{-1}.

Cu(L-A)(L-BH) ———; Cu(D-A)(L-BH)- - - - -.

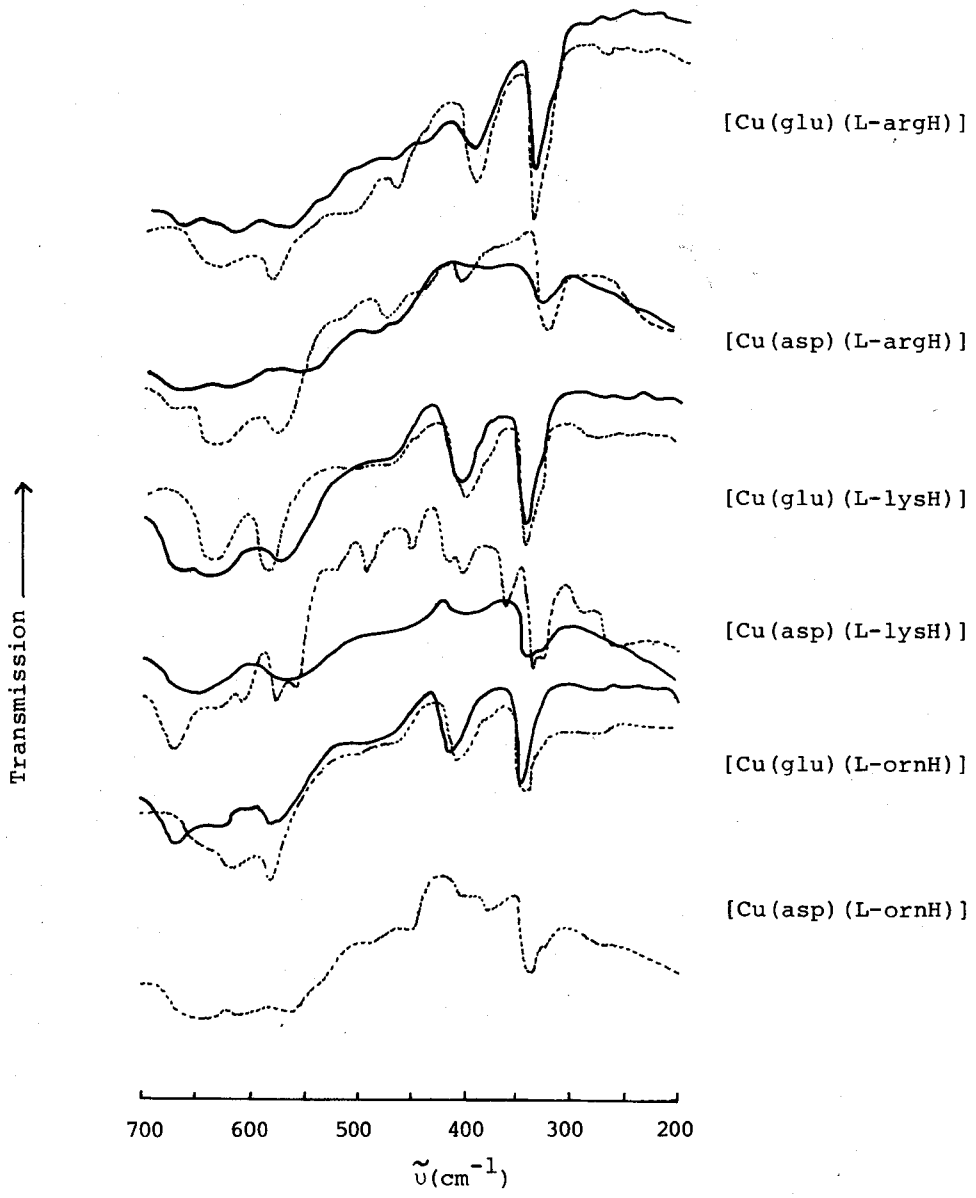


Figure 3. Infrared Spectra of Copper(II) Complexes in the Range 700-200 cm^{-1}.

Cu(L-A)(L-BH)———; Cu(D-A)(L-BH)·····.

ious pH values between 5 and 11 were satisfactorily reproducible. The spectral data are summarized in Table 2 and some examples are depicted in Figures 4 and 5(solid lines). The CD magnitudes of the ternary systems increase with pH, indicating that the mixed ligand complexes are the major species present at pH 7-10. The systems with Orn show marked spectral changes at pH > 8 owing to the apical coordination by the δ-amino group,[8,9] while the other systems including those with Asp show moderate changes at this pH.

On the assumption that the CD magnitude is an additive function under the present conditions, the theoretical CD curves for the ternary systems(broken lines in Figures 4 and 5) were estimated at every wavelength by summing up half the magnitudes($1/2\Delta\varepsilon$) of CuA_2 and $Cu(BH)_2$. As is apparent from Figure 4, the L-valine- or L-alanine-containing ternary systems, where no intramolecular ligand-ligand interaction is expected, exhibit excellent fits of the calculated curves to the experimental ones. On the other hand, serious deviations of the curves are observed most remarkably for the systems with two L-ligands both of which have an oppositely charged side chain that can form intramolecular electrostatic bondings(Figure 5). Since the spectra were obtained for 5×10^{-3} M solutions, these observations may imply that such discrepancies arise from the intramolecular bonds, which cause fixation of the complex structures and reduce the freedom of motion of side chains. Regarding the Cu-D-AH_2-L-BH_2 systems,

Table 2. Relative CD Magnitude in Water (I=variable)[a)]

System	pH	$\Delta\varepsilon_{max}$ (Found)	Relative magnitude	λ_{max} Found	λ_{max} Calcd[b)]
Cu-L-Val-L-Arg	7.2	-0.19	1.0	590	590
	8.6	-0.19	1.0	580-590	590
Cu-L-Val-L-Glu	7.4	-0.19	1.0	600	600
	8.8	-0.19	1.0	600	600
Cu-L-Ala-L-Arg	9.4	-0.10	1.0	610	600
Cu-L-Ala-L-Glu	7.8	-0.10	1.0	610	610
Cu-L-Ala-L-Asp	9.3	-0.06	1.0	630	630
Cu-L-Glu-L-Arg	7.3	-0.14	1.4	600	610
	8.5	-0.15	1.3	600	600
Cu-L-Glu-L-Lys	7.3	-0.13	1.2	600	610
	9.0	-0.13	1.1	600	600
Cu-L-Glu-L-Orn	7.5	-0.13	1.4	600	600
Cu-L-Asp-L-Arg	7.2	-0.09	1.4	630	630
	9.5	-0.09	1.3	630	630
Cu-L-Asp-L-Lys	7.2	-0.08	1.2	640	630-640
	9.6	-0.08	1.1	640-650	630-640
Cu-L-Asp-L-Orn	7.1	-0.07	1.5	640	640

a) Relative CD magnitude refers to $\Delta\varepsilon_{max}$(found)/$\Delta\varepsilon_{max}$(calcd).

b) The approximate maximum wavelength estimated from the CD spectral curves.

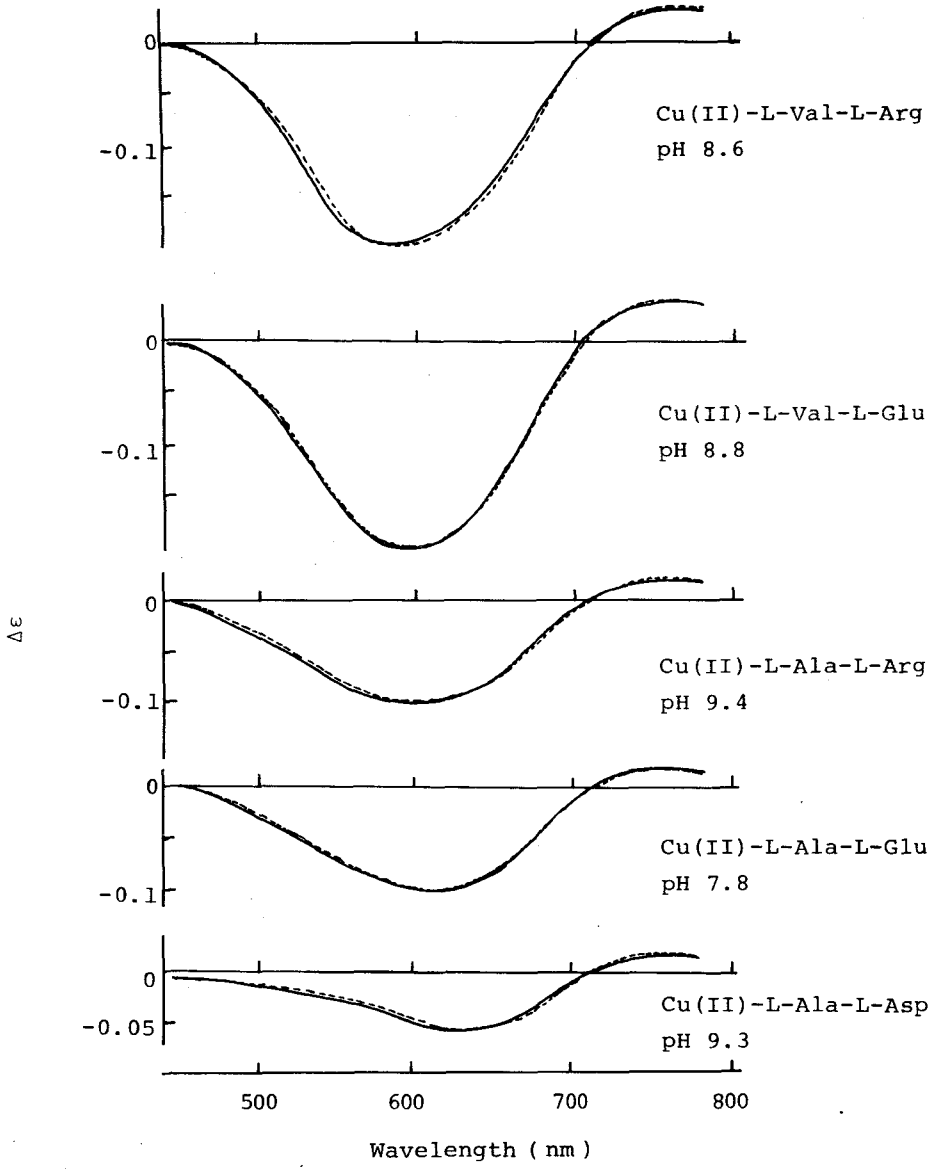


Figure 4. Circular Dichroism Curves of Ternary Copper(II) Systems without Electrostatic Ligand-Ligand Interactions. Experimental Curves: ———, Estimated Curves: - - - - - .

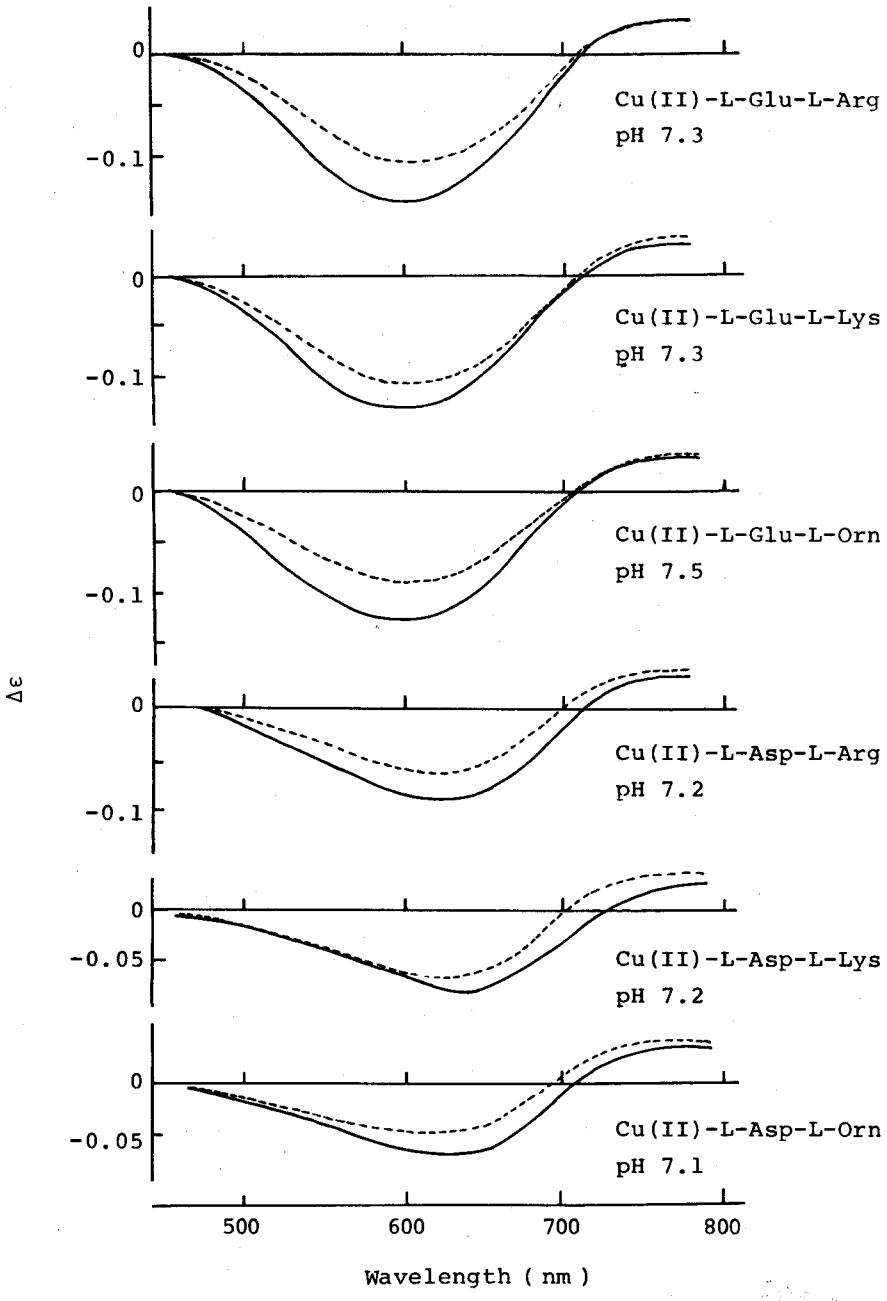


Figure 5. Circular Dichroism Curves of Ternary Copper(II) Systems Containing Ligands with Oppositely Charged Side Chains.

Experimental Curves: ——— , Estimated Curves: - - - - - - .

the experimental curves do not deviate greatly from the expected ones. Because the absolute values of $\Delta\varepsilon$ for these systems are much smaller than those for the Cu-L-AH_2-L-BH_2 systems and the deviations are roughly in the range of experimental errors, it is difficult to find significant differences between them.

Inspection of the curve fit of the L-Orn-containing systems was made at pH 7-8, because, as mentioned earlier, they exhibit spectral changes at pH>8 due to apical coordination. Anomalous CD curves of these systems will be briefly referred to later.

The observed CD magnitude may be explained by the hexadecant(D_{4h}) rule,[10,11] according to which the side chains of the L-ligands in [Cu(L-A)(L-BH)] with the *trans* geometry 1 are located in the sectors with negative sign, and this configuration may contribute to the observed magnitude enhancement. Although the complex formation in the binary systems is supposed to be nearly complete at pH 9-10[12] from the pH dependence of the CD magnitude, the above argument is contingent on the species distribution in the ternary systems at equilibrium, and a more quantitative comparison of the CD magnitudes will be made from the study of the solution equilibria(Chapter 3).

The absorption spectral data in the visible region are listed in Table 3, which indicates that the peaks of the ternary systems are between those of the corresponding binary systems. The maximum wavelengths and the ε values do not

Table 3. Absorption Spectal Data

System	pH	λ_{max} (nm)	ε	Remarks
Cu-L-Glu-L-Arg	5.2	670	36	
	7.3	625	57	
	8.5	622	56	
Cu-D-Glu-L-Arg	5.4	651	55	
	7.3	626	58	
	9.4	(620) a)	(68) a)	turbid
Cu-L-Glu-L-Lys	5.8	677	35	
	7.3	626	53	
	9.0	(622)	(68)	turbid
Cu-D-Glu-L-Lys	5.5	659	39	
	7.4	625	52	
	9.3	(623)	(56)	turbid
Cu-L-Glu-L-Orn	5.2	660	39	
	7.5	625	56	
	9.2	626	59	
Cu-D-Glu-L-Orn	5.5	643	44	
	7.8	620	57	
	9.3	626	59	
Cu-L-Asp-L-Arg	5.3	(622)	(40)	cryst b)
	7.2	633	53	
	9.5	(630)	(55)	ppt c)
Cu-D-Asp-L-Arg	5.5	(654)	(38)	cryst
	7.3	634	53	
	9.8	(641)	(54)	ppt
Cu-L-Asp-L-Lys	5.3	659	37	
	7.2	632	50	
	9.6	630	52	
Cu-D-Asp-L-Lys	5.7	651	40	
	7.4	633	49	
	9.7	(629)	(55)	turbid
Cu-L-Asp-L-Orn	7.1	630	51	
	9.4	630	54	
	10.8	634	56	
Cu-D-Asp-L-Orn	7.3	634	51	
	9.4	630	54	
	10.8	636	57	

a) Numbers in parentheses are approximate values.

b) Crystals separated.

c) A precipitate was formed.

explicitly reflect the effects of the ligand-ligand interactions and differences due to the L- and D-enantiomers, although there are observed slight disagreements in the shapes of the curves. This may not be unexpected, because all the chelate rings of the systems in consideration have the same structures around the copper(II) ion and the ε values of the absorption spectra are more than a hundred times greater than the $\Delta\varepsilon$ values of the CD spectra.

As regards α-amino acids as ligands, only a small number of mixed ligand copper(II) complexes have so far been reported,[13] and this may be taken as an indication that isolation of such labile complexes is not always feasible under normal conditions but requires additional factors favoring or stabilizing ternary systems. CD spectral information strongly suggests the existence of ligand-ligand bondings and leads to the conclusion that such intramolecular bondings serve as an effective driving force for the mixed ligand complex formation.

The δ-amino group of ornithine is known to form an apical bonding with copper(II).[8,9] Figure 6 illustrates the pH dependence of their spectra, whose numerical data are summarized in Table 4. Since aspartate binds with copper(II) ion as a terdentate ligand whose β-carboxylate group is located at the apical position,[14] the spectral behaviors suggest that L-Asp and possibly L-Glu compete with the δ-amino group of L-Orn for the apical coordination.

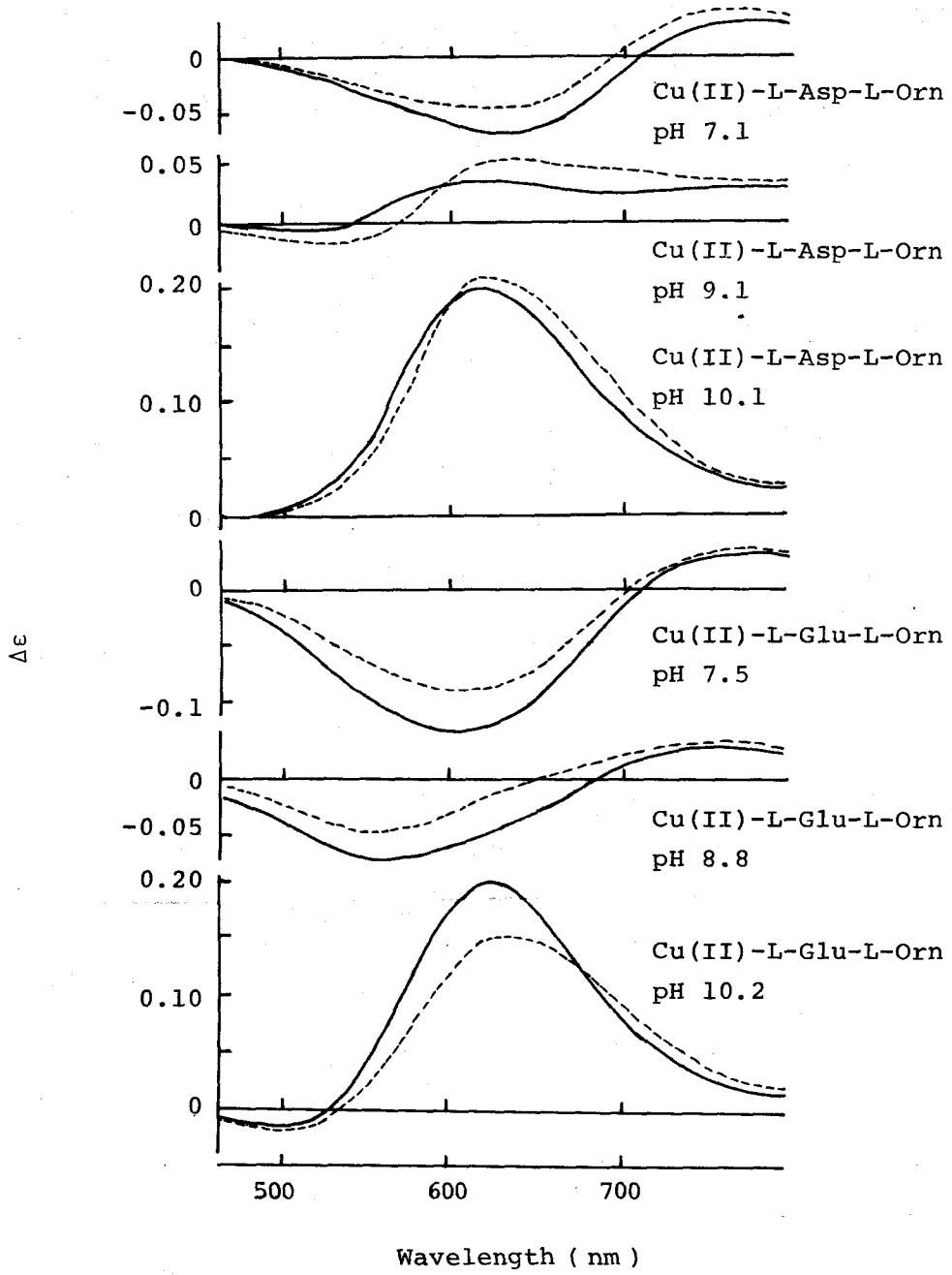


Figure 6. Circular Dichroism Spectra of the Systems Cu-L-Asp-L-Orn and Cu-L-Glu-L-Orn.

Experimental Curves: ———, Estimated Curves: - - - - - .

Table 4. Absorption and CD Spectral Data for the Systems Cu-L-Glu-L-Orn and Cu-L-Asp-L-Orn at Various pH Values (I=variable)

System	pH	CD				Absorption	
		Found		Estimated[a)]			
		$\Delta\varepsilon$	λ_{max} (nm)	$\Delta\varepsilon$	λ_{max} (nm)	ε	λ_{max} (nm)
Cu-L-Glu-L-Orn	7.5	-0.12_6	598	-0.09_0	604	55	626
	8.3	-0.10_0	590	-0.08_4	600	56	622
	8.8	-0.07_2	560	-0.04_6	554	58	624
	9.2	-0.04_5	538	-0.03_2	542	58	624
		$+0.03_6$	670	$+0.03_6$	740		
	9.5	$+0.15_7$	623	$+0.07_5$	631	57	627
	10.2	$+0.20_6$	623	$+0.15_6$	633	60	633
Cu-L-Asp-L-Orn	7.1	-0.07_1	630	-0.04_8	630	51	628
	9.4	$+0.03_6$	624	$+0.05_2$	630	54	631
	10.8	$+0.20_1$	618	$+0.21_0$	620	57	636

a) Estimated from the data for the corresponding binary systems.

Effects of Ionic Strength and Polarity of Solvent on the CD Magnitude

For the ternary systems with possible ligand-ligand interactions, the CD magnitudes expressed in terms of the relative magnitude, which assumes as unity the sum of the half magnitude($1/2\Delta\varepsilon$) exhibited by each of the optically active ligands in the complex, have been found to be larger than unity.[15] In accordance with the observation about the Cu-(edma)(L-BH), where edma refers to ethylenediamine-N-monoacetate,[15] the enhanced magnitude for the Cu-L-Glu-L-Arg system has now been shown to be affected by the ionic strength of solution and the polarity of the solvent used. The relative magnitude thus decreased from 1.3(I=variable) to 1.1(I=0.1) and 1.05(I=0.5)(Table 5). It might be suspected that the magnitude decrease at higher ionic strengths would result from the enhanced magnitudes for the binary systems to be compared. However, the molar extinction coefficients(ε) for the binary and ternary systems remain constant at different I values (Table 5), indicating that the observed decrease is not due to the shifts of the equilibria. It may therefore be inferred that the decrease is caused by the weakened ligand-ligand interactions at higher salt concentrations. The magnitude increase in 10, 20, and 50% aqueous ethanol confirms that the interactions are electrostatic attractions which are favored in non-aqueous solvents.

Table 5. Effects of Ionic Strength and Polarity of Solvent on the Absorption(AB) and CD Spectra for the Cu-L-Glu-L-Arg System

Medium[a]	Cu-L-Glu					Cu-L-Arg					Cu-L-Glu-L-Arg					Relative CD magnitude[b]
		CD		AB			CD		AB			CD		AB		
	pH	$\Delta\varepsilon$	λ_{max} (nm)	ε	λ_{max} (nm)	pH	$\Delta\varepsilon$	λ_{max} (nm)	ε	λ_{max} (nm)	pH	$\Delta\varepsilon$	λ_{max} (nm)	ε	λ_{max} (nm)	
water(I=var.)	8.5	-0.12_2	613	56	622	8.5	-0.11_1	598	56	622	8.5	-0.14_7	598	56	622	1.2_6
10% aq. ethanol	8.3	-0.12_3	612	59	619	7.9	-0.10_0	580	57	622	8.1	-0.15_0	601	59	621	1.3_4
20% aq. ethanol	8.2	-0.12_6	614	61	620	8.0	-0.10_1	580	58	620	8.5	-0.15_7	602	61	620	1.3_8
50% aq. ethanol	7.8	-0.13_8	616	62	620	8.1	-0.12_3	592	61	620	7.9	-0.17_3	600	63	620	1.4_4
water(I=0.1)	7.7	-0.11_9	612	57	622	7.7	-0.12_0	592	55	622	7.6	-0.13_4	599	57	622	1.1_2
10% aq. ethanol ($0.1M\ NaClO_4$)	8.7	-0.12_3	612	59	620	8.1	-0.12_0	586	56	622	8.3	-0.14_4	598	59	621	1.1_8
water(I=0.5)	7.8	-0.13_2	615	57	622	7.7	-0.14_1	590	56	622	7.7	-0.14_3	607	57	622	1.0_5

a) Ionic strengths were adjusted with $NaClO_4$. The pH values in aqueous ethanol are pH meter readings.

b) Relative CD magnitude refers to $\Delta\varepsilon_{max}$(found)/$\Delta\varepsilon_{max}$(calcd).

Chapter 3

Solution Equilibria of Ternary Copper(II)-Acidic Amino Acid(Aspartic Acid or Glutamic Acid)-Basic Amino Acid(Arginine, Lysine, or Ornithine) Systems

The synthetic and spectral studies described in the previous chapter have revealed that electrostatic ligand-ligand interactions serve as effective driving forces for formation of mixed ligand copper(II) complexes of α-amino acids. Since they can affect the free energy of complex formation, it is expected that the electrostatic interactions are quantitatively characterized by equilibrium constants of mixed ligand copper(II) complexes in solution. Formation constants of parent and mixed copper(II) complexes containing acidic amino acids(aspartic acid and glutamic acid) and basic amino acids(arginine, lysine and ornithine) have been measured potentiometrically at 25°C and ionic strength of I=0.1 (KNO_3). The intramolecular noncovalent ligand-ligand interactions have attracted much attention these few years as a novel type of the driving forces for the ternary complex formation.[15-17]

Experimental

Reagents

L- and D-aspartic acid(Asp, $aspH_2$), L- and D-glutamic acid (Glu, $gluH_2$), L-arginine(Arg, $argH_2$) hydrochloride, L-lysine (Lys, $lysH_2$) hydrochloride, L-ornithine(Orn, $ornH_2$) hydrochloride and L-alanine(Ala, alaH) were purchased from Nakarai Chemicals, Ltd. and dried *in vacuo* over P_4O_{10} before use. All other reagents used were of highest grade available. Water was distilled and deionozed.

Standard 0.1 M KOH was prepared according to Armstrong,[18] and stored under a nitrogen atmosphere. A stock solution (0.01 M) of $Cu(NO_3)_2$ was prepared by dissolving copper(II) nitrate trihydrate in water and standardized against standard zinc by chelatometric titration.

Apparatus

An Orion Research 801A Ion Analyzer with a 91-02-00 double junction reference electrode and a 90-01-00 glass electrode was used after standardization with Horiba standard buffer solutions(pH 4.01, 6.86 and 9.18) at 25°C.

Procedure

Titrations were carried out in water at 25 ± 0.05°C under a nitrogen atmosphere($I=0.1(KNO_3)$). The initial concentrations of the solutions to be titrated were 4×10^{-3} M for the determination

of acid dissociation constants of ligands, AH_2 and BH_2, and $2x10^{-3}$ M for Cu^{2+} and $4x10^{-3}$ M for AH_2 or BH_2 for the determination of stability constants of binary complexes. Solutions ($2x10^{-3}$ M) of equimolar amounts of Cu(II), AH_2 and BH_2 were used for the determination of stability constants of ternary complexes. Each reactant was pipetted into the solution to be titrated from the stock solutions freshly prepared before use. In 30 ml of the solution in a jacketed beaker thermostated at 25±0.05°C were immersed two electrodes, a burette tip and an inlet for purified nitrogen gas. Under stirring with a magnetic stirrer, each 0.05 ml aliquot of 0.1 M KOH was introduced into the solution, and the pH of the solution was measured until all dissociable protons were neutralized.

Calculation of Stability Constants

The stability constants were calculated by analyzing the pH titration data with a computer using the program SCOGS for multi-reactant systems, which employs the conventional non-linear least-squares method.[19] In SCOGS, refinement is performed by minimizing the sum of the squares of the residuals in titer, $\sum_i R_i$, where for i th point R_i = (actual titer of base) - (titer calculated). The stability constant was expressed as log formation constant, $\log \beta_{pqrs}$, for the following equation,

$$pCu^{2+} + qA^{2-} + rB^{-} + sH^{+} \overset{\beta_{pqrs}}{\rightleftharpoons} Cu_pA_qB_rH_s^{2p-2q-r+s} \quad (1)$$

$$\beta_{pqrs} = \frac{[Cu_pA_qB_rH_s^{2p-2q-r+s}]}{[Cu^{2+}]^p[A^{2-}]^q[B^{-}]^r[H^{+}]^s} \quad (2)$$

where A^{2-}, B^{-} and H^{+} represent completely deprotonated form of acidic amino acid(asp, glu), completely deprotonated form of basic amino acid(arg, lys, orn) and proton, respectively. For clarity each species is represented as pqrs, where each numeric letter refers to the number of each reactant Cu^{2+}, A^{2-}, B^{-} and H^{+} of the species in that order. Acid dissociation constants of α-amino group and amino group or carboxyl group in the side chain of each ligand were calculated using the computer program prepared by Yamauchi.[20] Ion product of water pKw' and the activity coefficient of H^{+} ion, f_H', were determined by titrating 0.1 M KNO_3 and 0.01 M nitric acid, respectively, with 0.1 M potassium hydroxide under the same conditions. The values of pKw' = 13.896 and f_H' = 0.865 were used in the calculations.

Results and Discussion

Acid Dissociation Constants of Ligands

Prior to determination of the stability constants of ternary complexes, the acid dissociation constants of ligands and the stability constants of binary complexes should be accurately determined. Acid dissociation constants of functional groups of α-amino acids were determined by using the computer program prepared by Yamauchi[20] and expressed in the form of the overall stability constants of each ligand species, $\log \beta_{pqrs}$, which were in good agreement with the reported values[21] (Table 5).

Stability Constants of Binary Copper(II) Complexes

Stability constants of binary complexes were calculated by non-linear least-squares refinement using the computer program SCOGS from the fixed stability constants of free ligand species which had been determined previously. Table 6 shows the values for the complexes containing acidic amino acids(Asp and Glu), which are similar to those determined under a differnt ionic strength(I=0.2(KCl)).[22] The calculations converged satisfactorily with only the 1101, 1100 and 1200 species excluding 1202 and 1201(the standard deviations expressed in titre were smaller than 0.05). Figure 7 clearly demonstrates the distributions of these binary species plotted against pH.

Table 6. Log Stability Constants of Ligands

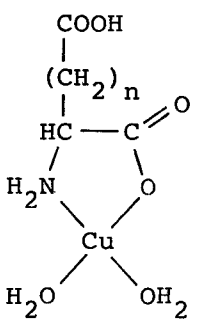
Ligand	Species			
	0101	0102	0011	0012
L-Asp	9.752	13.543		
D-Asp	9.791	13.556		
L-Glu	9.611	13.759		
D-Glu	9.688	13.894		
L-Arg			12.106	21.180
L-Lys			10.853	19.979
L-Orn			10.521[a)]	19.270[a)]

a) Taken from Reference 23.

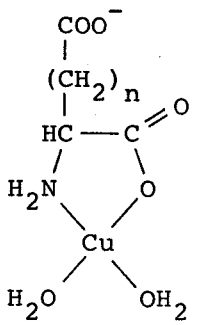
Table 7. Formation Constants for Binary Copper(II) Complexes Containing Acidic Amino Acids at 25°C and I=0.1(KNO_3); Standard Deviations Are Shown in Parentheses

AH_2	Species		
	1101	1100	1200
L-Asp	12.720(0.009)	8.997(0.004)	15.841(0.010)
D-Asp	12.696(0.009)	8.969(0.004)	15.813(0.009)
L-Glu	12.498(0.003)	8.304(0.001)	14.797(0.003)
D-Glu	12.492(0.005)	8.316(0.003)	14.858(0.005)

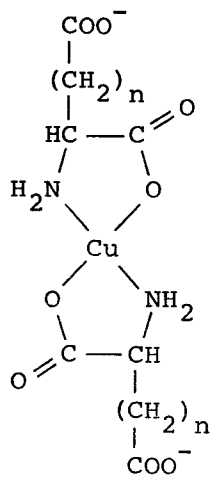
1101



1100



1200



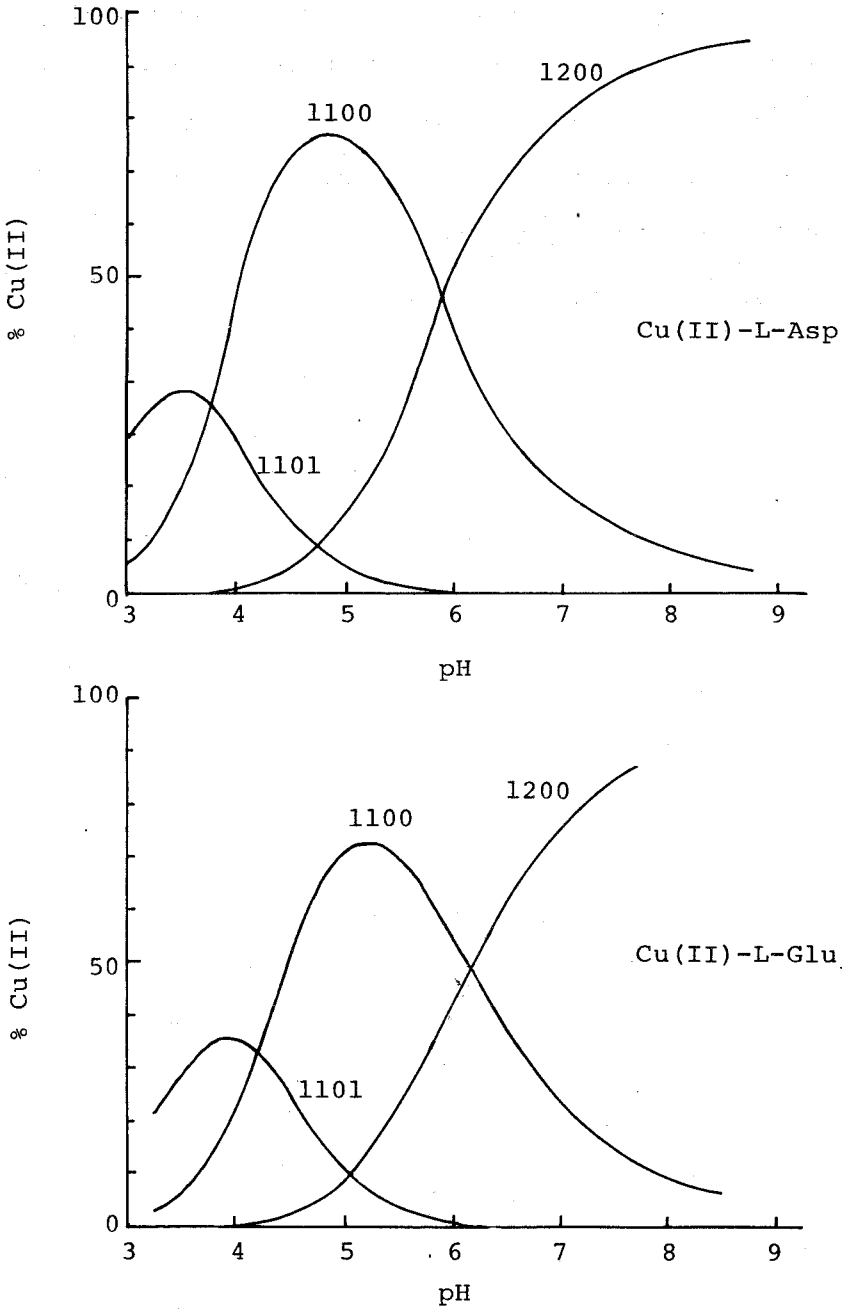


Figure 7. Species Distribution in Cu-L-AH_2 System as a function of pH at 25±0.05°C and I=0.1(KNO_3) (C_{Cu} = 0.002016 M, C_{AH_2} = 0.004000 M).

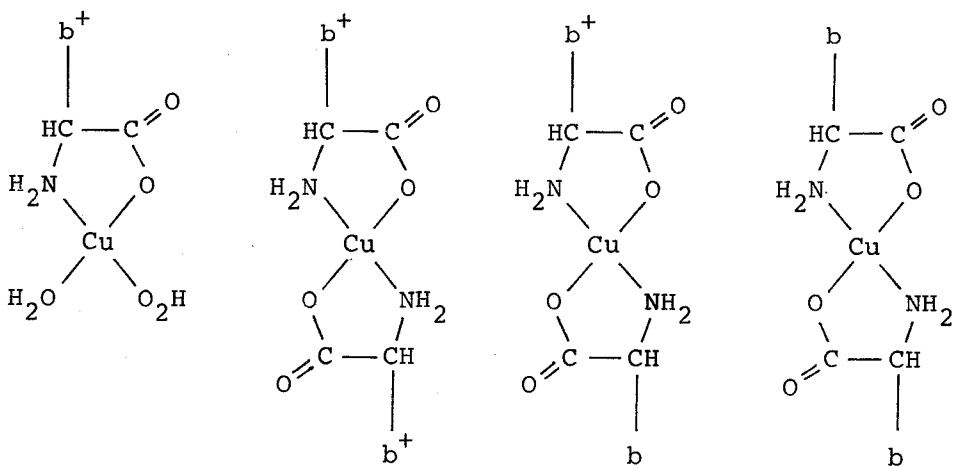
Stability constants for the binary complexes containing basic amino acids, Arg, Lys and Orn, and their species distribution curves are given in Table 8 and Figure 8, respectively (the standard deviations expressed in titre were below 0.005 for the Lys- and Orn-containing systems and *ca.* 0.05 for the Arg-containing systems). For Arg-containing systems, only the 1022 and 1011 species were observed and the 1021 and 1020 species with deprotonated guanidinium group in the side chain of Arg were not detected even in strongly alkaline solution.[23] On the other hand, for Lys- and Orn-containing systems, the 1021 and 1020 species, which carry one and two deprotonated ammonium groups in the side chain, respectively, appeared in the alkaline region besides the 1022 and 1011 species.

The residual stability constant, $\log \beta_{1022} - \log \beta_{1021}$, which exactly corresponds to the proton association constant of the functional group in the side chain of Lys and Orn incorporated into the binary complex, was greatly lowered for the Orn-containing system through the facile apical coordination of δ-amino group to the central copper(II) ion. However, $\log \beta_{1021} - \log \beta_{1020}$ was not remarkably lowered, suggesting that the other δ-amino group is probably not involved in the apical coordination. This coincides well with the peculiar CD spectral behavior of the Cu-L-Orn system.[24] For Lys-containing system, no prominent lowering of proton association constants of the ε-amino group was observed, because it is not apically coordinated to the copper(II) ion.

Table 8. Formation Constants for Binary Copper(II) Complexes Containing Basic Amino Acids at 25°C and I=0.1(KNO_3); Standard Deviations Are Shown in Parentheses

BH_2	Species			
	1011	1022	1021	1020
L-Arg	19.55(0.06)	37.91(0.08)	——	——
L-Lys	18.458(0.007)	35.631(0.008)	25.639(0.010)	15.072(0.008)
L-Orn	17.948(0.006)	34.649(0.007)	25.730(0.008)	15.706(0.008)

1011 1022 1021 1020



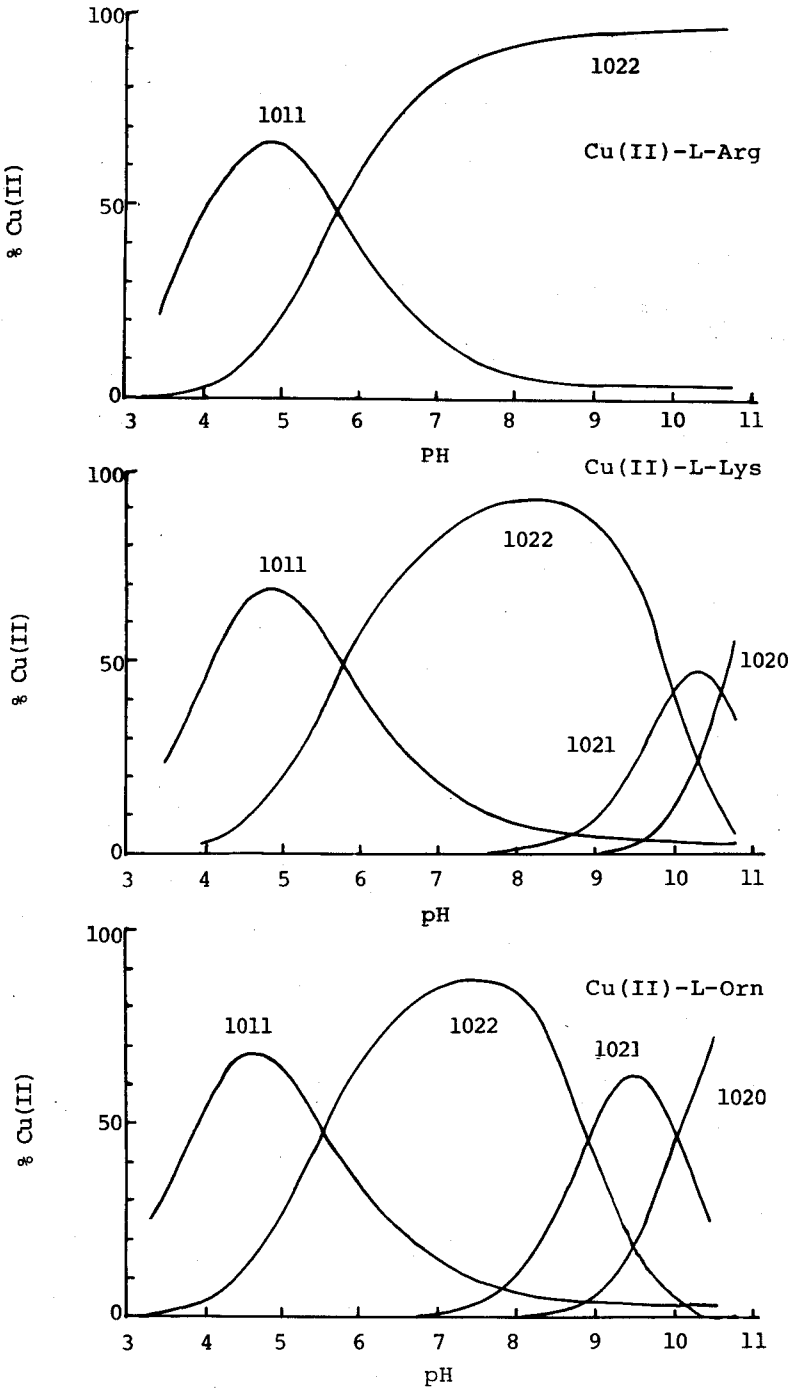


Figure 8. Species Distribution in Cu-L-BH_2 System as a Function of pH at 25±0.05°C and I=0.1(KNO_3) (C_{Cu} = 0.002016 M, C_{BH_2} = 0.004000 M

Stability Constants of Ternary Copper(II) Complexes

The stability constants of the ternary complexes were calculated in an analogous manner with the computer program SCOGS,[19] using the previously determined equilibrium constants of the binary complexes and ligands. Table 9 shows those for the systems containing L- and D- Asp and L- and D-Glu as acidic amino acids(AH_2) and L-Arg, L-Lys and L-Orn as basic amino acids(BH_2)(standard deviations expressed in titre were *ca.* 0.01). For the Arg-containing systems, because of the tight association of proton to the guanidino group in the side chain, the only ternary species 1111 with possible electrostatic ligand-ligand interactions including hydrogen bondinings, appears both in neutral and alkaline solutions, and the deprotonated species 1110 did not appear even at high pH values. On the other hand, for the Lys- and Orn-containing systems the 1110 species were also detected in alkaline solution through the deprotonation from the ammonium group of the 1111 species.

The proton association constants of the amino groups in the side chains of Lys and Orn incorporated into the ternary complexes are expressed by the differences, $\log \beta_{1111} - \log \beta_{1110}$, which were generally somewhat larger than those of the corresponding binary complexes(Table 10). Moreover, the values were much larger than those of the ternary systems containing L-Ala instead of Asp and Glu and thus losing the possibility of ligand-ligand interactions. The depression of the deprotonation from the ammonium group in particular ternary systems

Table 9. Formation Constants for Ternary Copper(II) Complexes at 25°C and I=0.1(KNO_3); Standard Deviations Are Shown in Parentheses

		Species	
BH_2	AH_2	1111	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-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)	——

Table 10. Effect of Intramolecular Electrostatic Ligand-Ligand Interaction on Acid Association of Side Chain Amino Group

BH_2	AH_2	$\log \beta_{1111} - \log \beta_{1110}$	$\log \beta_{1022} - \log \beta_{1021}$	$\log \beta_{1021} - \log \beta_{1020}$
L-Lys	L-Asp	10.50	9.99 (9.93)[a]	10.57 (10.47)[a]
L-Lys	L-Glu	10.52		
L-Lys	L-Ala	10.00		
L-Orn	L-Asp	10.36	8.92	10.02
L-Orn	L-Glu	10.25		
L-Orn	L-Ala	9.49		

a) Taken from Reference 23.

is most reasonably ascribed to the expected electrostatic ligand-ligand interactions with the carboxylate group of Asp or Glu.

The species distribution curves for the copper(II)-L-Asp-BH_2 systems(Figure 9) show the predominant formation of the ternary species 1111 in the neutral region. The distribution curves for the copper(II)-L-Glu-BH_2 systems shown in Figure 10 also established the preferential formation of the 1111 species in the same region. Accordingly, it is confirmed that the CD spectral magnitude enhancements observed only for the systems with possible ligand-ligand interactions(Chapter 2) are due to the increased asymmetry around the central copper(II) ion through such interactions.

Statistically expected stability constants for the deprotonated species 1110 were estimated according to the following equation:

$$\log \beta_{1110\,(calcd)} = 1/2(\log \beta_{1200} + \log \beta_{1020}) + \log 2 \qquad (3)$$

where log 2 is the statistical factor. Comparison of the stability constants with the corresponding calculated values (Table 11) shows slight destabilization of the 1110 species, suggesting the interaction between the amino group and the carboxylate group in contrast to the systems containing L-Ala in place of L-Asp and L-Glu. This may indicate that the interaction is not so strong as to sufficiently compensate the strain on both ligands due to the ligand-ligand interactions.

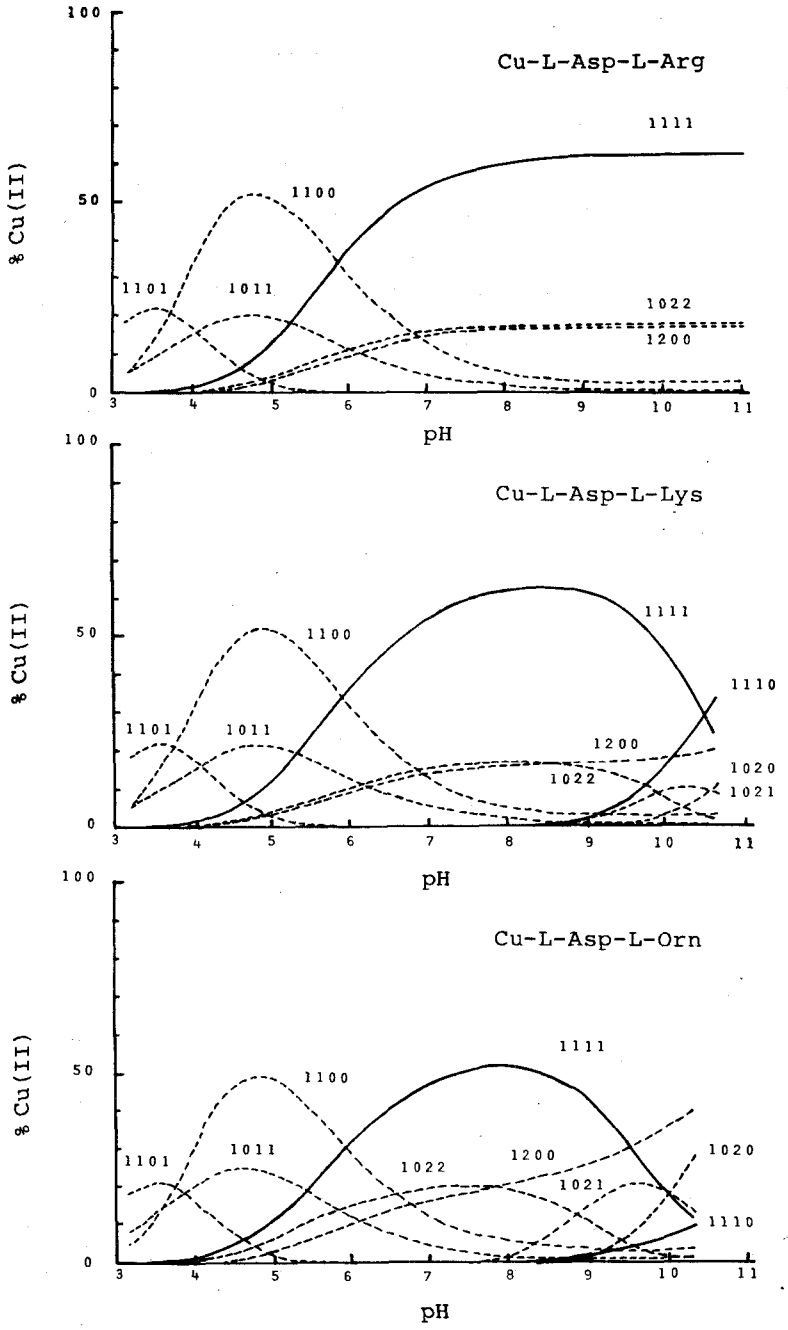


Figure 9. Species Distribution in Cu-L-Asp-L-BH_2 System as a Function of pH at 25±0.05°C and I=0.1(KNO_3) (C_{Cu} = 0.002016 M, C_{Asp} = 0.002000 M, C_{BH_2} = 0.002000 M).

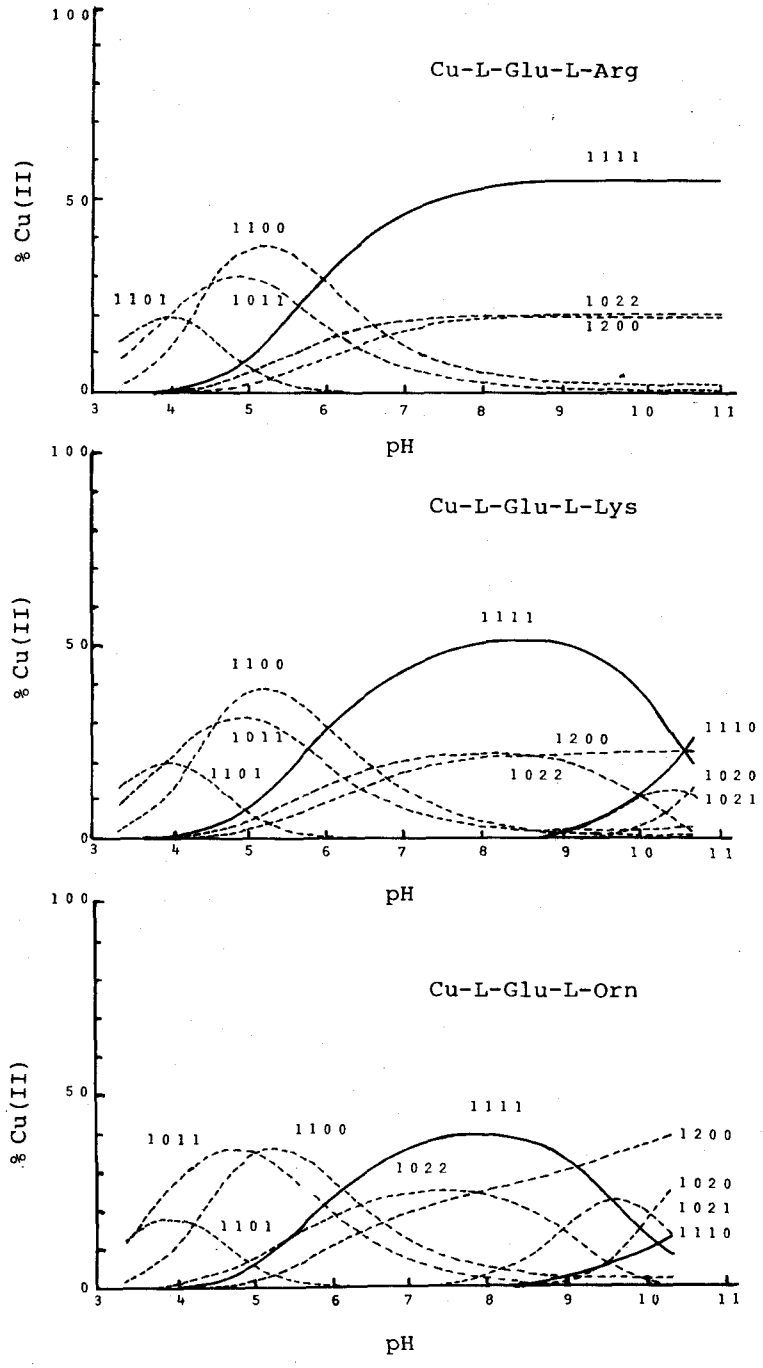


Figure 10. Species Distribution in Cu-L-Glu-L-BH_2 System as a Function of pH at 25±0.05°C and I=0.1(KNO_3) (C_{Cu} = 0.002016 M, C_{Glu} = 0.002000 M, C_{BH_2} = 0.002000 M).

Table 11. Comparison of the Stability Constants with the Statistically Estimated Values.

BH_2	AH_2	Species 1110	1110[a] (calcd)	1111	1111[b] (calcd)
L-Arg	L-Asp	——	——	27.43	27.18
L-Arg	L-Glu	——	——	26.61	26.66
L-Lys	L-Asp	15.82	15.76	26.32	26.04
L-Lys	L-Glu	15.08	15.24	25.60	25.52
L-Lys	L-Ala	15.67	15.26	25.67	25.53
L-Orn	L-Asp	15.29	16.08	25.65	25.55
L-Orn	L-Glu	14.68	15.56	24.93	25.03
L-Orn	L-Ala	15.53	15.57	25.02	25.04

a) $1/2(\log \beta_{1020} + \log \beta_{1200}) + \log 2.$

b) $1/2(\log \beta_{1022} + \log \beta_{1200}) + \log 2.$

Similar comparison for Orn-containing systems seems to be of limited significance because of the uncertainty of the estimated values due to the tridentate coordination of Orn.

When the estimated values for the 1111 species are given by the following equation,

$$\log \beta_{1111\,(calcd)} = 1/2(\log \beta_{1200} + \log \beta_{1022}) + \log 2 \qquad (4)$$

and compared with the corresponding stability constants, the stabilization of the 1111 species is apparent: the observed values for the 1111 species of some systems are greater than the estimated values, and for the Cu-L-Glu-L-Orn system, the the difference $\log \beta_{1111} - \log \beta_{1111\,(calcd)}$ is larger than $\log \beta_{1110} - \log \beta_{1110\,(calcd)}$, although the values are negative.

The reduction of ionic strength from 0.1 to 0.033 for the Cu-L-Glu-L-Lys system caused the predominant enhancement of log stability constants of the ternary species, i. e. from 25.60 to 25.92 for the 1111 species and from 15.08 to 15.85 for the 1110 species(statistically estimated values are 25.65 and 15.50, respectively).[25] Although the populations of the ternary species are greater at lower ionic strength and the stability enhancement is significant, attention should be paid to the simple comparison of the values obtained at different conditions because stabilities of all other species present are also affected to some extent. Nevertheless, it seems apparent that reduction of ionic strength increases the

stability of the ternary complexes through the reinforcement of ligand-ligand interactions under favorable conditions for electrostatic bondings. Such a trend in the stability also explains the anomalous CD spectral behaviors described in the previous chapter.

All the active complexes [Cu(L-A)(L-BH)] and the *meso* complexes [Cu(D-A)(L-BH)](llll species) and [Cu(L-A)(L-B)] and [Cu(D-A)(L-B)](lll0 species) had similar stabilities, which agree with each other to within the experimental errors(Table 8). This indicates that the ligand-ligand interaction which gives rise to geometrical isomerism in the active and *meso* form complexes are not so effective as to effect stereoselectivity in aqueous solution.

In addition to the synthetic and spectral approaches, the approach through solution equilibria also indicated the existence of intramolecular electrostatic ligand-ligand interactions in the ternary copper(II) complexes composed of acidic and basic amino acids carrying oppositely charged groups in their side chains. Although the steric requirements associated with the intramolecular ligand-ligand interactions are apt to destabilize the ternary complexes with such interactions,[16f] it is substantiated that the electrostatic interactions are finally able to overcome the adversity, and stabilize the ternary complex, which is often observed in biological systems.

Chapter 4

Preparation and Spectral Properties of Mixed Copper(II) Complexes Containing L-Histidine

Human blood serum contains approximately 1 μg of copper per ml. Although a large portion of it is unexchangeably bound to an α_2-globulin, ceruloplasmin, 5 to 10 percent of copper in serum is bound to albumin and this portion is in extremely rapid equilibrium with copper in tissues. This rapid equilibration occurs *via* low molecular weight ligands for copper which can effectively compete for the binding of copper with albumin. It was established that these low molecular weight ligands are amino acids existing in large excess over copper at normal levels. In addition, ultrafiltration and ultracentrifugation studies suggested that most important complexes as copper carrier may be the ternary ones containing L-histidine and L-threonine, L-glutamine, or L-asparagine.[26-30]

Because of their occurrence and involvement in biological systems, histidine-containing ternary amino acid-copper(II) complexes have attracted wide attention.[17,31-34] In fact, L-histidinato-L-threoninatocopper(II) was detected in human blood serum,[26] and its monohydrate was later revealed by X-ray crystal analysis to have His coordinated as a terdentate ligand (Figure 11).[35]

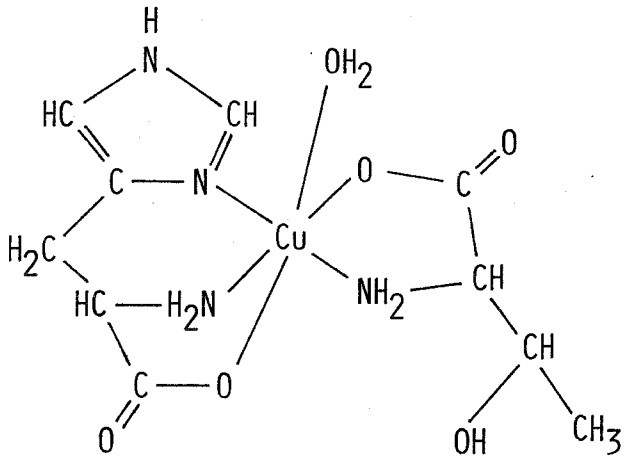


Figure 11. Structure of L-Histidinato-L-threoninatoaquacopper(II) Complex(Taken from Reference 35).

In the previous chapter electrostatic ligand-ligand interactions within complex molecules have been shown to lead to the ternary complex formation and fix the complex in a particular configuration. It is interesting to note that threonine, glutamine and asparagine offer polar groups that can interact with each other around copper(II) and that the molecular models suggested the existence of intramolecular ligand-ligand interactions between the polar groups. This may offer useful clues to understanding why histidine preferentially forms ternary copper(II) complexes with some limited amino acids, such as threonine, glutamine and asparagine, as low molecular weight copper carriers in blood serum. The driving force for such preferential formation of the ternary species has hitherto remained unrevealed.

Experimental

Materials

L-Histidine(His, $hisH_2$) hydrochloride, L- and D-asparagine(Asn, asnH), L-glutamine(Gln, glnH), L-threonine(Thr, thrH), L-serine(Ser, serH), L-alanine(Ala, alaH), L-valine (Val, valH) and glycine(Gly, glyH) were purchased from Nakarai Chemicals, Ltd. and D-histidine from Protein Research Foundation. For pH titrations, A grade L- and D-histidine hydrochloride, L-asparagine, L-glutamine, L-threonine and L-serine were purchased from Ajinomoto Chemical Co. All other chemicals used were of highest grade available.

Preparation of Ternary Complexes

(a) Synthesis of [Cu(L-his)(L-asn)] in two modifications

L-Histidine hydrochloride(0.95 g, 5 mmol), L-asparagine (0.75 g, 5 mmol) and copper(II) perchlorate hexahydrate(1.85 g, 5 mmol) were dissolved in *ca.* 30 ml of water, and the pH of the solution was adjusted at 7.5-8.0 with aqueous sodium hydroxide. After stirring for 1 h at room temperature the solution was concentrated *in vacuo* and filtered. By adding methanol or ethanol to this solution, the complex [Cu(L-his)(L-asn)]·1/4 H_2O was obtained as blue prismatic crystals. On the other hand, crystallization from aqueous solution gave blue needles of [Cu(L-his)(L-asn)]·$4H_2O$. Analytical data are shown in Table 12.

(b) Synthesis of [Cu(L-his)(D-asn)] in two crystal modifications

Synthetic attempts using equimolar amounts of Cu^{2+}, L-His and D-Asn always gave the binary complex $Cu(D\text{-}asn)_2$ as crystals. However, by using a 1 : 1.3 : 0.9 molar ratio, the ternary complex [Cu(L-his)(D-asn)] was isolated as crystals with 3 and 1.5 H_2O of crystallization from aqueous ethanol with a low and a high ethanol content, respectively.

(c) Synthesis of other ternary complexes containing L-histidine

Isolation of active and *meso* form histidine-containing ternary complexes with other amino acids were tried and those depicted in Table 12 were isolated as crystals. Conditions for isolation were included in the Table.

Measurements

Infrared, absorption and circular dichroism spectra were measured according to the procedures similar to those described in Chapter 2.

Potentiometric titrations were performed for ternary copper(II) systems containing L-histidine and L-asparagine, L-glutamine, L-threonine, L-serine, glycine, L-alanine and L-valine according to the procedure as described in Chapter 3. The acid dissociation constants of each ligand and the stability constants of the binary complexes were taken from the literature.[21]

Table 12. Elemental Analyses of Histidine-Containing Ternary Copper(II) Complexes

Complex	Formula	Elemental Analysis Found(%) C	H	N	Calcd(%) C	H	N	Condition for Preparation Cu:his:aa	Solv.(H_2O:EtOH)
[Cu(L-his)(L-asn)]·0.25H_2O	$C_{10}H_{15}N_5O_5Cu \cdot 0.25H_2O$	34.09	4.57	19.93	33.99	4.42	19.82	1:1:1	aq. EtOH(1:1)
[Cu(L-his)(L-asn)]·4H_2O	$C_{10}H_{15}N_5O_5Cu \cdot 4H_2O$	28.37	5.52	16.58	28.54	5.51	16.64	1:1:1	water
[Cu(L-his)(D-asn)]·3H_2O	$C_{10}H_{15}N_5O_5Cu \cdot 3H_2O$	29.50	5.31	17.44	29.82	5.26	17.39	1:1.3:0.9	aq. EtOH(1:1)
[Cu(L-his)(D-asn)]·1.5H_2O	$C_{10}H_{15}N_5O_5Cu \cdot 1.5H_2O$	31.96	4.67	18.64	31.96	4.83	18.64	1:1.3:0.9	aq. EtOH(1:4)
[Cu(D-his)(L-gln)]·2H_2O	$C_{11}H_{17}N_5O_5Cu \cdot 2H_2O$	32.85	4.95	17.71	33.12	5.31	17.56	1:1:1	aq. EtOH(1:4)
[Cu(L-his)(L-thr)]·2H_2O	$C_{10}H_{16}N_4O_5Cu \cdot 2H_2O$	32.00	5.43	15.07	32.30	5.42	15.07	1:1:1.3	aq. EtOH(1:4)
[Cu(D-his)(L-thr)]·1.5H_2O	$C_{10}H_{16}N_4O_5Cu \cdot 1.5H_2O$	33.35	5.23	15.12	33.10	5.28	15.44	1:0.9:1.3	aq. EtOH(1:4)
[Cu(L-his)(L-ser)]·4H_2O	$C_9H_{14}N_4O_5Cu \cdot 4H_2O$	27.60	5.51	14.35	27.45	5.63	14.23	1:1:1	aq. EtOH(1:1)
[Cu(L-his)(L-ser)]·H_2O	$C_9H_{14}N_4O_5Cu \cdot H_2O$	31.71	4.80	16.54	31.81	4.75	16.49	1:1:1	aq. EtOH(1:4)

Results and Discussion

Preparation and Characterization of [Cu(L-his)(asn)]

L-Asparaginato-L-histidinatocopper(II) has been isolated as crystals in different forms, i. e. as deep blue prismatic crystals from aqueous methanol or ethanol and as blue needles from water with 0.25 and 4 molecules of water of crystallization, respectively. The IR spectral pattern of the former was entirely different from that of the latter, which on dehydration at 60°C *in vacuo* gave the identical IR spectral pattern with the former(Figure 12). This suggests that both modifications have slightly different conformations of the side chains of ligands affected by water molecules of crystallization. Their somewhat similar IR spectral patterns in the region of 700-200 cm^{-1}(Figure 13) further support this view and indicate that both forms have similar structures around the central copper(II) ion. On the other hand, [Cu(L-his)(D-asn)]·$3H_2O$ and [Cu(L-his)(D-asn)]·$1.5H_2O$ exhibited quite different IR spectral patterns in the region of 4000-200 cm^{-1}(Figures 12 and 13), which might point out the existence of geometric isomerism similar to 1 and 2 or very different structures around the central copper(II) ion.

X-ray crystal structure analyses[36] showed the absence of the expected intramolecular ligand-ligand interactions between the amide group of asparagine and the carboxylate group of histidine in [Cu(L-his)(L-asn)] with different crystal struc-

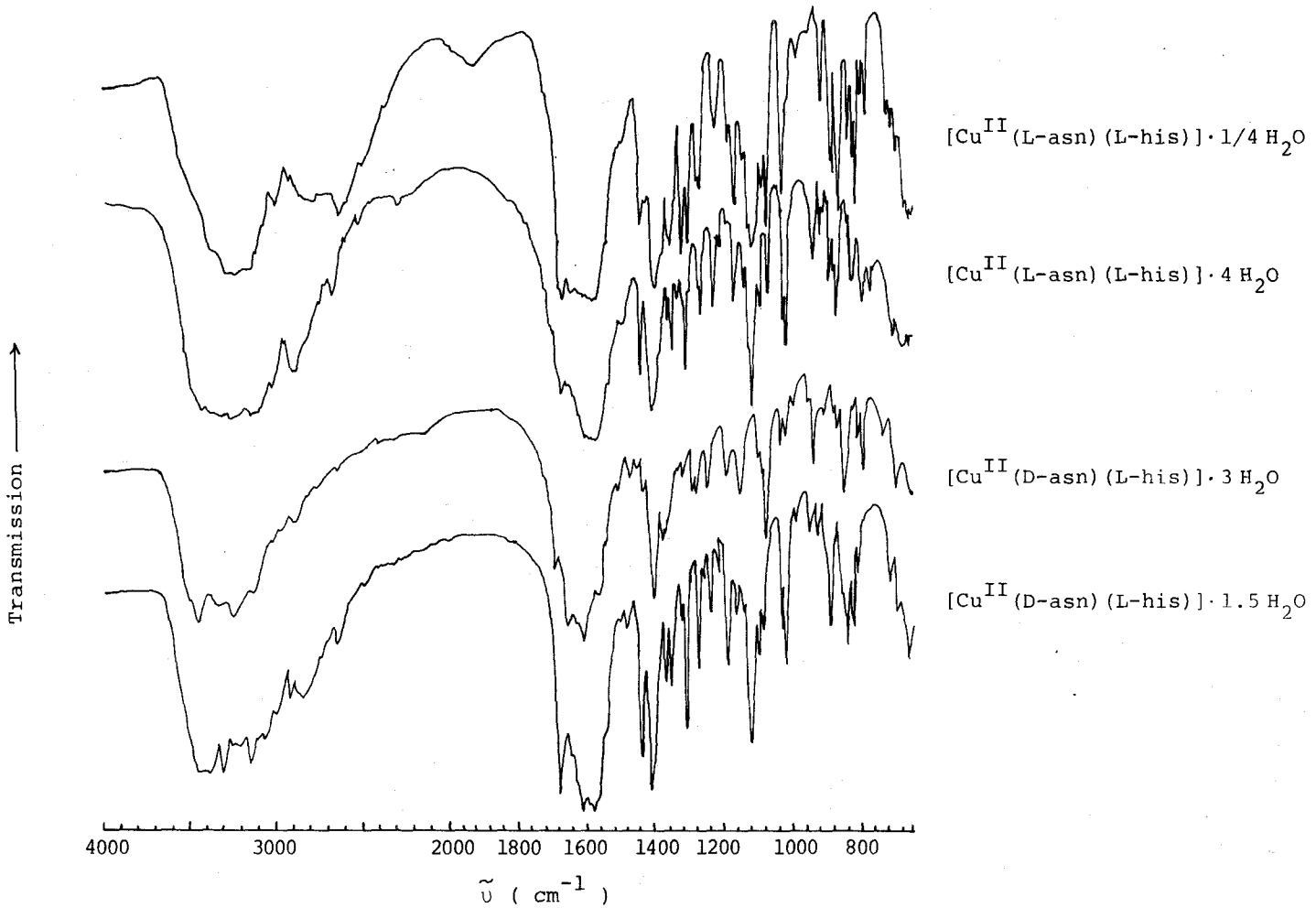


Figure 12. Infrared Spectra of $[Cu^{II}(asn)(L\text{-}his)]$ in the Range 4000-650 cm^{-1}.

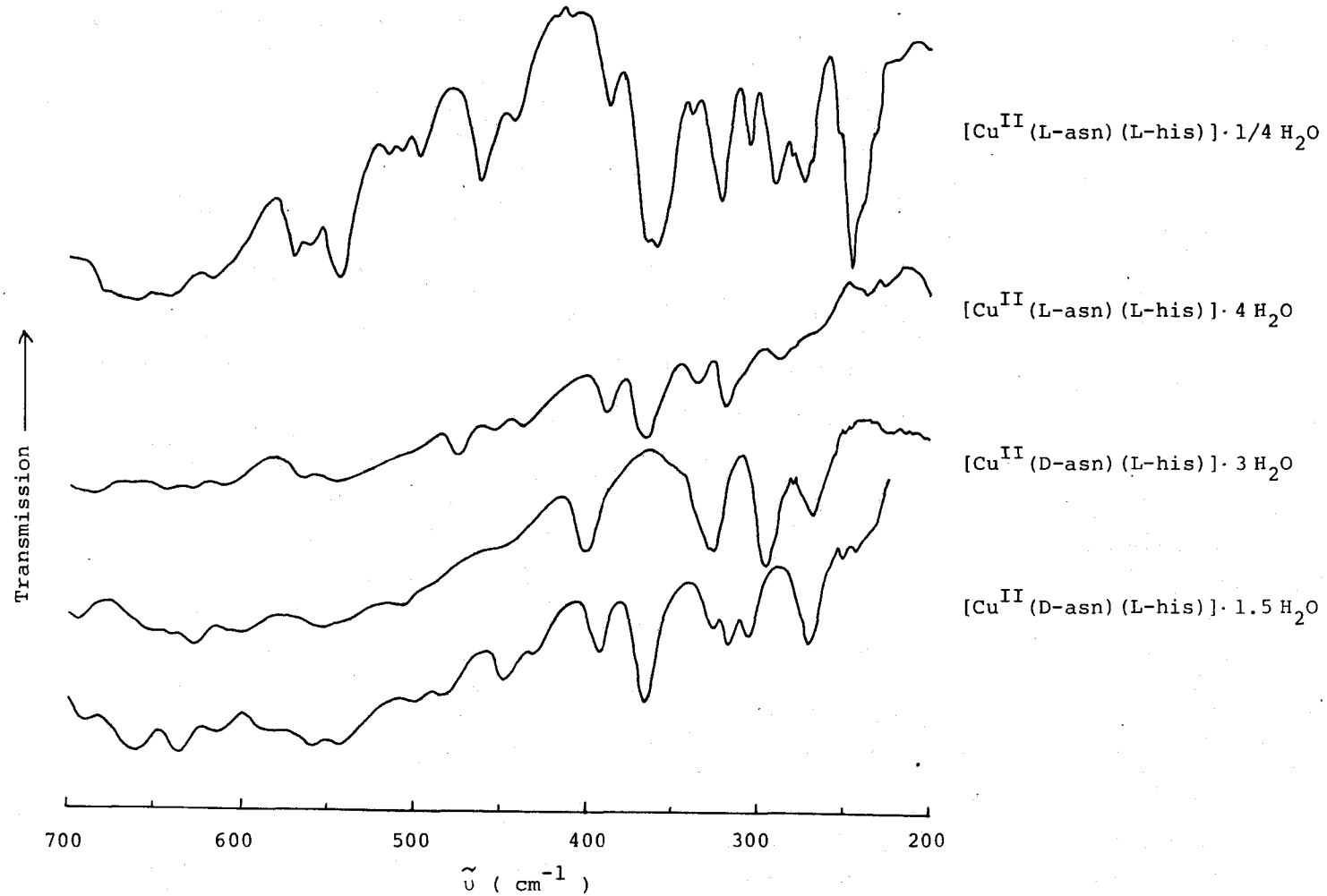


Figure 13. Infrared Spectra of $[Cu^{II}(asn)(L\text{-}his)]$ in the range 700-200 cm^{-1}.

tures (3 and 4 of Figures 14 and 15). Although in the crystalline state the amide group is hydrogen-bonded to a neighboring complex and assists the growth of the crystal, in solution it will interact directly or indirectly through an intervening water molecule with the carboxylate group of histidine at the apical position on the same side of the square plane of coordination with the *cis* arrangement. An intramolecular interaction is improbable in aqueous solution, because water molecules existing in great excess separate each complex molecule. If the C-C bond of $-CH_2-CONH_2$ in 4 rotates about 180°, the expected direct interaction is quite easily attained!

A ternary complex containing a nucleotide, Cu(II)-α,α'-bipyridine(BIPY)-inosine 5'-monophosphate(IMP), serves as an example showing the difference between the proposed structure in aqueous solution and the structure found in the solid state. The existence of the stacking interaction between BIPY and the base moiety of IMP was suggested through the detection of a charge transfer band, and coordination of the two nitrogens of BIPY and the two oxygens of the phosphate group of IMP was proposed[16a] (Figure 16). On the contrary, X-ray crystal structure analysis[37] revealed the completely unlike structure (Figure 17), i. e. IMP coordinates *via* N(7) of inosine moiety and a water molecule occupies the rest of the coordination sites, exhibiting the absence of the predicted stacking.

The information on structures of ternary copper(II) complexes in aqueous solution is available from spectroscopic

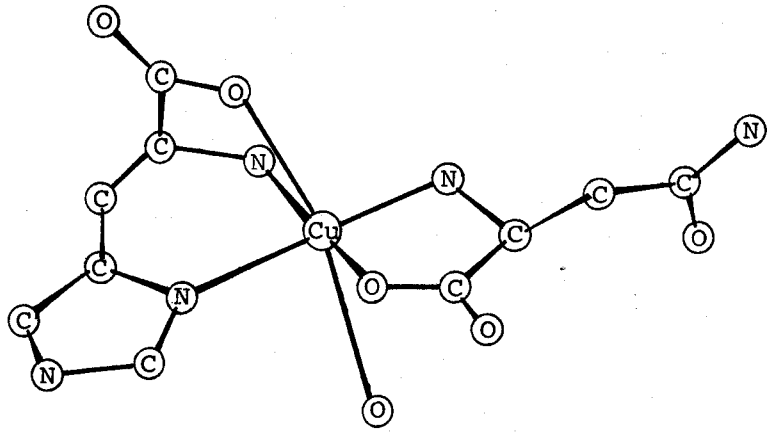


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Figure 14. Structure of L-Asparaginato-L-histidinatoaquacopper(I trihydrate Complex.

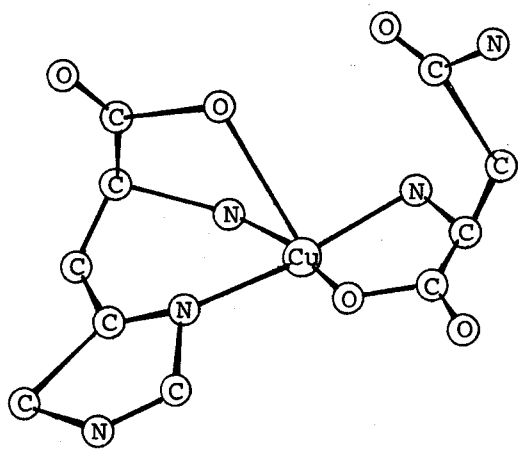

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Figure 15. Structure of L-Asparaginato-L-histidinatocopper(II) Complex.

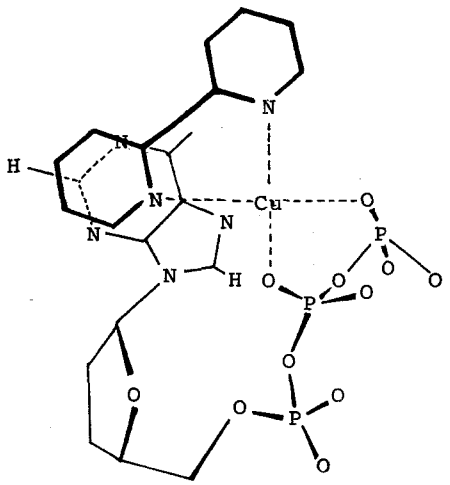

Figure 16. Tentative and Simplified Structure for the Ternary Complex Formed between Cu(α,α'-bipyridyl)$^{2+}$ and ATP^{4-} (Taken from Reference 16a). Cu(BIPY)(IMP) was supposed to have a similar structure.

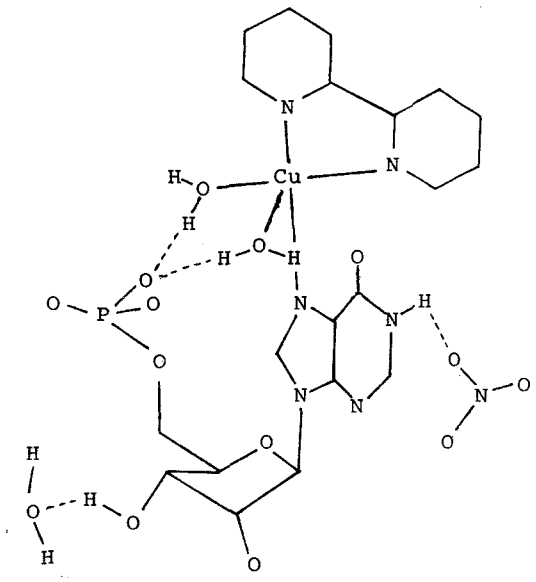

Figure 17. The Molecular Structure of [Cu(BIPY)(IMP)]·NO_3·H_2O (Taken from Reference 37). Broken Lines Represent the Hydrogen Bonds.

measurements of aqueous solutions and studies on solution equilibria. Calorimetric study indicated that asparaginate either serves as bi- and/or terdentate ligand to copper(II) by weekly locating its $CONH_2$ group in the vicinity of one of the long axial bonds of Cu(II) or gives a mixture of bi- and terdentate asparaginate complexes.[33] This supports the probable interaction of the $CONH_2$ group of asparagine with the carboxylate group of histidine located at the apical position.

Other Histidine-Containing Ternary Copper(II) Complexes

Ternary complexes of [Cu(D-his)(L-gln)], [Cu(L-his)(L-thr)], [Cu(D-his)(L-thr)] and [Cu(L-his)(L-ser)] were isolated as crystals. Other active or *meso* form complexes and the ternary complexes with glycine, alanine, valine, leucine, arginine, 2,4-diaminobutyric acid, aspartic acid, glutamic acid, etc. were not isolated as crystals, but only oily products or binary complexes were obtained. Successful isolation of histidine-containing ternary complexes with only those amino acids which have amide or hydroxyl groups would be originated from the crystal propagation assisted by the intermolecular hydrogen bondings pertaining to these groups. Inspection using space-filling models points out that direct or indirect intramolecular interactions as inferred in [Cu(his)(asn)] can reasonably exist in these ternary complexes. Outer-sphere coordination between Cu^{2+} and the hydroxy-oxygen of serine *via* water[38] also suggests the probable interaction

between the carboxylate group of histidine and the hydroxyl group of serine.

Absorption and Circular Dichroism Spectra

Absorption spectra of ternary systems composed of copper(II), L-histidine and other amino acids exhibited similar λ_{max} and ε_{max} values in the region of 610-620 nm at neutral pH values(Table 13), suggesting that all ternary complexes contain the structures around the central copper(II) ion similar to those of [Cu(L-his)(L-thr)] and [Cu(L-his)(L-asn)] (Figures 11, 14 and 15).

CD spectra for these systems showed marked positive peaks around 630 nm through the electronic effect,[39,40] and the magnitudes were more prominent than expected acoording to the similar procedures described in Chapter 2. A rough proportionality was recognized between the CD magnitude differences between the systems Cu-L-His-glycine and Cu-L-His-L-amino acid and the magnitudes of binary systems Cu-L-amino acid(Figure 18). Interestingly, the points corresponding to the systems containing neutral amino acids with an alkyl group in the side chain, such as L-alanine and L-valine, are on the straight line. On the other hand, the points corresponding to the systems containing amino acids with a functional group in the side chain randomly deviate from the line, probably demonstrating perturbations of the CD spectra due to direct or indirect interactions of the functional group of the relevant amino acids with the car-

Table 13. Absorption and CD spectral Data of Ternary Systems Containing L-Histidine and Other L-Amino Acids

Cu-L-His-L-aa System		Absorption		CD	
Ligand aa	pH	λ_{max}	ε	λ_{max}	$\Delta\varepsilon$
Gly	7.5	620	53	630	+0.29
L-Ala	7.6	610	57	620	+0.28
L-Val	7.2	610	58	620	+0.25
L-Thr	7.4	610	56	640	+0.26
L-Ser	7.5	610	56	630	+0.24
L-Gln	7.2	610	57	620	+0.28
L-Asn	7.3	610	57	620	+0.29
L-His	8.0	644	85	686	+0.39

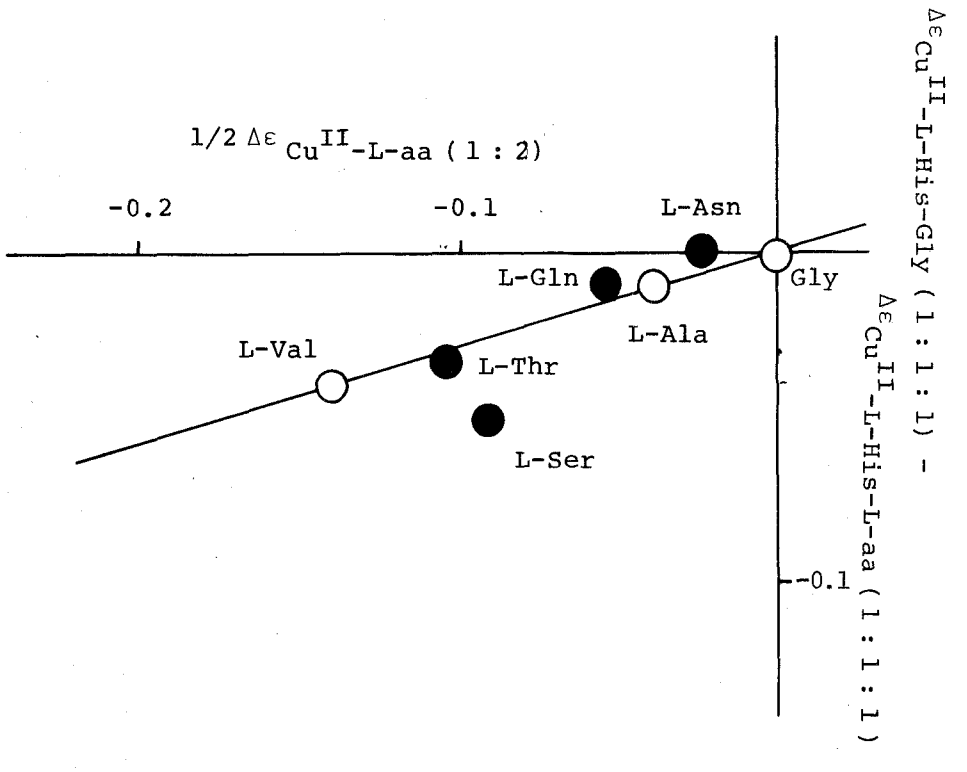

Figure 18. Effects of Possible Ligand-Ligand Interactions on CD Specra.

boxylate group of histidine coordinated at the apical position.

Solution Equilibria of Histidine-Containing Ternary Systems

The stability constants(expressed in logarithm form) of ternary copper(II) complexes containing other amino acids were calculated by analyzing pH titration data at 25±0.05°C and ionic strength of 0.1(KNO_3) with the use of a computer program SCOGS(Table 14). It is apparent that the ternary complex(1110 species) is the most predominant species in the neutral pH region as typically depicted by the species distribution curves of the system copper(II)-L-histidine-L-asparagine(Figure 19).

Coordination of an aromatic nitrogen in pyridine or imidazole is known to stabilize the complex through its π-back-bonding.[41] The stability constants obtained according to the procedure described in Chapter 3 show that all histidine-containing ternary copper(II) complexes with Gly, L-Ala, L-Val L-Thr, L-Asn, L-Gln and L-Ser have greater stabilities than expected ones. Although the existence of noncovalent ligand-ligand interactions seems apparent from the CD spectral study, the interactions do not contribute significantly to the stabilization of the ternary complexes under the conditions used; the extent of stabilization, which may be inferred from the $\log \beta - \log \beta_{calcd}$ values for all the ternary complexes, is not solely interpreted in terms of the ligand-

Table 14. Formation Constants of Ternary Copper(II) Complexes at 25°C and I=0.1(KNO_3); Standard Deviations Are Given in Parentheses

Complex	log β	log β_{calcd} [a]	Δlog β [b]
[Cu(L-his)(gly)]	17.40(0.02)	16.94	0.46
[Cu(L-his)(L-ala)]	17.24(0.02)	16.76	0.48
[Cu(L-his)(L-val)]	17.31(0.03)	16.74	0.57
[Cu(L-his)(L-thr)]	17.08(0.02)	16.70	0.38
[Cu(L-his)(L-ser)]	17.18(0.02)	16.63	0.55
[Cu(L-his)(L-asn)]	17.03(0.01)	16.56	0.47
[Cu(L-his)(L-gln)]	17.06(0.02)	16.45	0.61

a) $\log \beta_{calcd} = 1/2(\log \beta_{Cu(L\text{-}his)_2} + \log \beta_{Cu(L\text{-}aa)_2}) + \log 2$.

b) $\Delta\log \beta = \log \beta - \log \beta_{calcd}$.

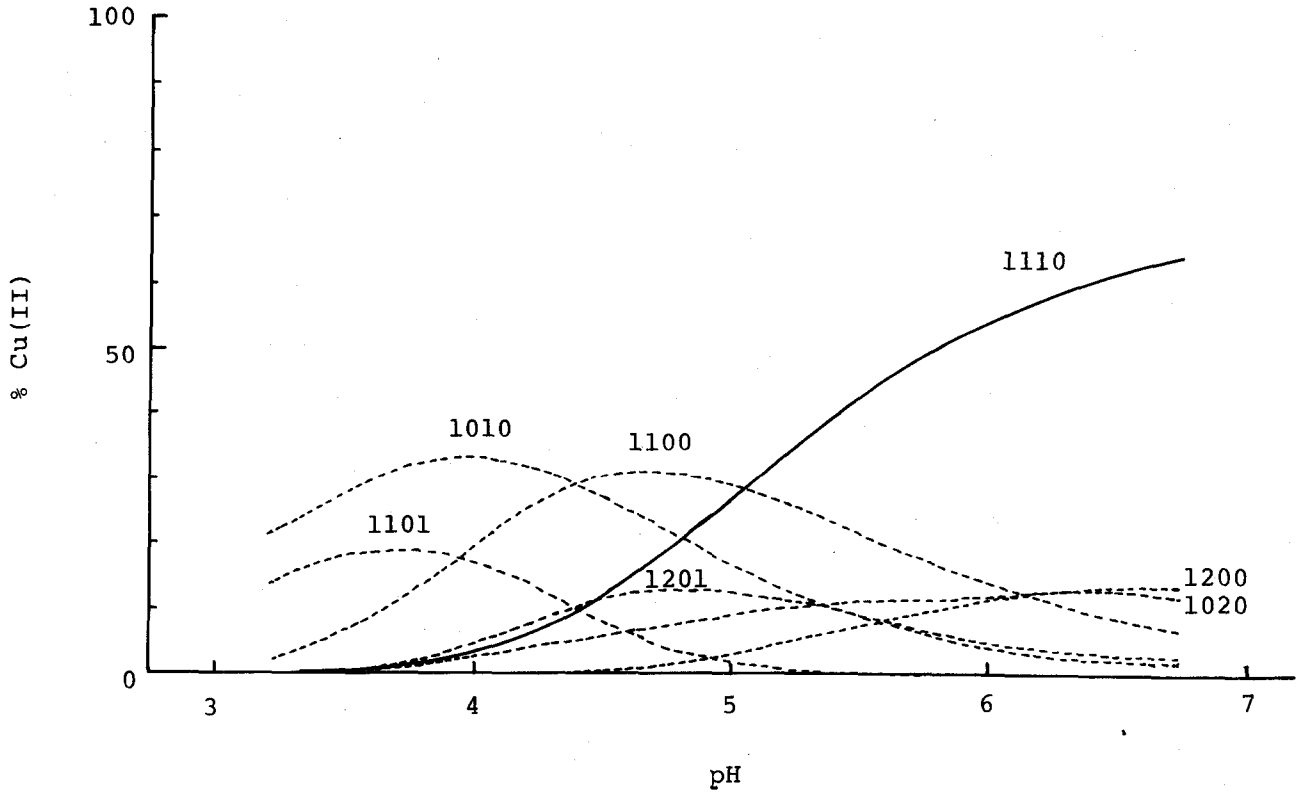

Figure 19. Species Distribution in Cu-L-His-L-Asn System as a Function of pH at 25±0.05°C and I=0.1(KNO_3) (C_{Cu} = 0.002016 M, $C_{L\text{-}His}$ = 0.002000 M, $C_{L\text{-}Asn}$ = 0.002000 M).

ligand interactions. So it is concluded that the stabilization of the histidine-containing ternary complexes is mainly due to the coordination of the imidazole group to copper(II) ion, whereas the ligand-ligand interactions play only a minor role for the ternary complex formation in aqueous solution.

Histidine-Containing Ternary Copper(II) Complexes as Copper Carriers in Blood Serum

The ligand-ligand interactions may not be so favorable under physiological conditions at an elevated temperature(37°C) and a higher ionic strength(I=*ca.* 0.15). The recent computer simulation study by May et al.[42] suggested that most copper(II) ions are bound in Cu(cystinate)(histidinate) and Cu(histidinate)$_2$ and a small portion in the other histidine-containing ternary complexes. However, in serum, proteins including albumin, amino acids, organic acids such as citrate and lactate, and inorganic substances such as carbonate and phosphate, may alter the character of liquid greatly to assist the noncovalent interactions and thus finally stabilize the ternary complexes such as [Cu(L-his)(L-thr)] through the ligand-ligand interaction. Nevertheless, Sarkar et al.[26] and Sass-Kortsak et al.[28] seem to have exaggerated the importance of histidine-containing ternary copper(II) complexes as low molecular weight copper carriers. Other binary and ternary complexes should not be excluded! The histidine-containing ternary copper(II)

complexes with compact structures and higher stabilities based on the ligand-ligand interactions might be regarded as transient transport forms specifically effective in the membrane rather than as general copper carriers in serum.

Chapter 5

Optical Resolution of α-Amino Acids *via* Mixed Ligand Copper(II) Complex Formation

Stereoselectivity has been reported recently for kinetically labile transition metal complexes containing simple amino acids,[34,38,43-45] and N-carboxymethyl[46] and N-benzyl derivatives[47] of amino acids, and in most cases the selectivity has been attributed to the steric hindrance arising from two bulky ligands coordinated around a metal ion. Therefore, the selectivity attributable to steric interactions between the side chains of ligands in the coordination sphere has been applied to optical resolution of amino acids with a bulky side chain by ligand-exchange chromatography in the presence of copper(II) and other metal ions.[48] Since the selectivity is purely due to steric hindrance, resolvable amino acids have been confined to those having a bulky group, such as proline, valine, and leucine.

The ligand-ligand interactions between the oppositely charged groups in the side chains of coordinated amino acids have been inferred from the CD spectral magnitude enhancements in the d-d region(Chapter 2) and solution equilibrium study (Chapter 3). They have been interpreted as a driving force leading to the formation of mixed ligand copper(II) complexes.

Because of the steric requirements for such interactions around the central atom, they were expected to give rise to geometric isomerism in the ternary copper(II) complexes, Cu(A)(BH): a *trans* structure 1 for Cu(L-A)(L-BH) and a *cis* structure 2 for Cu(D-A)(L-BH), where A refers to asp, or glu and B to arg, lys, or orn. So it is expected that amino acids are optically resolved *via* stereoselective isolation of ternary complexes due to the geometric isomerism. It is further expected that one enantiomer of histidine may be preferentially incorporated into the ternary complex, Cu-(his)(L-aa), where aa refers to asn, gln, thr or ser, on the basis of the difference between the systems Cu-L-his-L-aa and Cu-L-his-D-aa(Chapter 4), which again is probably due to the geometric isomerism

Experimental

Materials

D-Arginine hydrochloride and L-lysine hydrochloride were obtained from Fluka AG and D-ornithine hydrochloride from Sigma Co. All other amino acids were purchased from Nakarai Chemicals, Ltd. Their purities were checked by specific rotations, $[\alpha]_{589}^{20}$(in 3 M HCl; c=1), which were +25.7°(L-Asp), -24.9°(D-Asp), +31.9°(L-Glu), -30.9°(D-Glu), +22.8°(L-Arg·HCl), -21.7°(D-Arg·HCl), +20.8°(L-Lys·HCl), -20.5°(D-Lys·HCl), +22.9°(L-Orn·HCl) and -22.5°(D-Orn·HCl). The values for L-His·HCl·H_2O and D-His·HCl were +9.1° and -9.8°(in 6 M HCl; c=1), respectively. All other reagents used were of highest grade available.

Measurements

Optical rotations of amino acids were measured on a Yanagimoto Direct-reading polarimeter OR-10 at 589 nm in a 5-cm glass cell 20±0.1°C. All other measurements were similarly carried out as described in the preceding chapters.

Optical Resolution of Racemic Aspartic and Glutamic Acids

For every pair of DL-AH_2 and L-BH_2, optical resolution of an acidic amino acid was performed by essentially the same method as typically described for the Cu(II)-DL-Asp-L-Arg

system. Copper(II) perchlorate hexahydrate(3.70 g, 10 mmol), DL-aspartic acid(2.66 g, 20 mmol), and L-arginine hydrochloride(2.10 g, 10 mmol) were dissolved in *ca.* 50 ml of water, and the pH of the resulting solution was adjusted to *ca.* 7 with aqueous hydroxide. After stirring for 1 h at room temperature, the reaction mixture was concentrated *in vacuo* to a small volume at temperatures below 50°C. Addition of ethanol to the residue gave $[Cu(asp)(L\text{-}argH)]\cdot 2H_2O$ as blue crystals(0.68 g, 1.7 mmol; 17% based on the amount of copper(II) used). Found: C, 29.29; H, 5.51; N, 17.27%. Calcd for $C_{10}H_{19}N_5O_6Cu\cdot 2H_2O$: C, 29.66; H, 5.73; N, 17.30%

After copper(II) had been removed by treating an aqueous solution of the isolated complex with hydrogen sulfide, the incorporated aspartic acid was separated from L-arginine through a 1x100 cm column of Dowex CCR-2(mesh 20-50) in the H^+ form by eluting with water. Isolated aspartic acid was recrystallized from aqueous ethanol to give a salt-free product(0.16 g, 1.2 mmol; 12% based on the half amount of DL-aspartic acid used). The specific rotation of -22.9° (in 3 M HCl; c=1) demonstrates that D-aspartic acid was preferentially incorporated into the ternary complex. Its optical purity(89%) was substantiated by an estimation made from the CD curve of the isolated ternary complex in aqueous solution(pH 8.0) according to the method described below.

In order to determine the optical purities of the acidic amino acids incorporated into [Cu(asp)(L-lysH)], [Cu(asp)-

(L-ornH)], and [Cu(glu)(L-ornH)], the first crops, which were nearly pure, were recystallized from aqueous ethanol.

Optical Resolution of Racemic Arginine, Lysine and Ornithine

According to a procedure very similar to that described above, racemic basic amino acids were resolved into enantiomers by using a different Cu(II) : L-AH_2 : DL-BH_2 molar ratio of 1 : 1 : 1.5 to avoid precipitation of less soluble binary complexes $Cu(BH)_2(ClO_4)_2$. The amino acids BH_2 incorporated into Cu(L-A)(BH) were separated from L-AH_2 through a 1x100 cm column of Amberlite IR-45 in the Cl^- form by eluting with water and obtained as hydrochloride.

Determination of Optical Purity by CD Spectral Curves

The optical purities of the incorporated amino acids were also determined by the CD calibration curves, which were based on either the magnitude or the maximum wavelength and set up for the Cu(A)(L-BH) and Cu(L-A)(BH) systems with five different enantiomer contents of AH_2 and BH_2, respectively, at the molar ratio of 1 : 1 : 1. The measurements were made at selected pH values where the complex formation was nearly complete. The enantiomer contents of the Cu(glu)(L-BH) and Cu(L-A)(BH) systems were found to be linearly correlated with the CD magnitudes($\Delta\varepsilon$) at fixed wavelengths around 600 nm. Because the magnitudes for the Cu(asp)(L-BH) systems changed only slightly at different L- or D-Asp contents, the calibra-

tion curves were made by plotting the enantiomer contents against the maximum wavelengths that shifted with contents.

Optical Resolution of Histidine

$Cu(ClO_4)_2 \cdot 6H_2O$(3.7 g, 10 mmol), DL-histidine hudrochloride(2.87 g, 15 mmol) and L-asparagine(1.5 g, 10 mmol) were dissolved in *ca.* 30 ml of water. The pH of the resulting solution was adjusted to *ca.* 7 with dilute aqueous sodium hydroxide. On standing overnight, it gave fine blue needles (1.03 g, 25%). Found: C, 29.03; H, 5.33; N, 17.08%. Calcd for $C_{10}H_{15}N_5O_5Cu \cdot 3.5H_2O$: C, 29.16; H, 5.38; 17.00%.

After copper(II) had been removed by treating an aqueous solution of the isolated complex with hydrogen sulfide, the incorporated histidine was separated from L-asparagine through a 1x100 cm column of Amberlite IR-45 in the Cl^- form by eluting with water and obtained as hydrochloride hydrate. The isolated histidine(His·HCl·H_2O) contained a small amount(*ca.* 4%) of inorganic impurities, which were not removed by recrystallization from aqueous methanol. The specific rotation $[\alpha]_{589}^{20}$ of +7.9°(in 6 M HCl, c=1) demonstrated that L-histidine was preferentially incorporated into the ternary complex. Its optical purity(84%) was substantiated by an estimation made from the CD curve of the isolated ternary complex in aqueous solution(pH *ca.* 7.3) at I=0.1(KNO_3) according to the same method as described above.

Crystallization of the ternary complex from aqueous

methanol gave prismatic crystals whose IR spectrum was identical with [Cu(L-asn)(L-his)]·0.25H_2O(Yield 0.89 g, 25% based on the amount of copper(II) used). Found: C, 33.70; H, 4.42; N, 19.72%. Calcd for $C_{10}H_{15}N_5O_5Cu·0.25H_2O$: C, 33.99; H, 4.42; N, 19.82% The histidine incorporated into the ternary complex was found to be almost exclusively the L-enantiomer(98%) by the method described above.

Results and Discussion

Stereoselective Incorporation of Asp, Glu, Arg, Lys and Orn into Ternary Complexes, [Cu(A)(L-BH)] or [Cu(L-A)(BH)]

Yields and optical purities of the acidic and basic amino acids obtained *via* the ternary complex formation are summarized in Tables 15 and 16. The specific rotations clearly indicated that the D-enantiomers of the racemic amino acids used were preferentially incorporated into the ternary copper(II) complexes each containing an L-AH_2 or an L-BH_2. Another line of evidence supporting the incorporation of the D-enantiomers is given by the optical purities of the AH_2 and BH_2 estimated directly from the CD spectra of the complexes in the d-d region by referring to the calibration curves such as are shown in Figures 20 and 21. The optical purities determined by the two methods are in reasonable agreement with each other, and the differences between the corresponding values may be due to the inaccuracies pertaining to the calibration curves based on the CD spectra. The IR spectra of the isolated complexes, [Cu(A)(L-BH)], showed the patterns that were more closely related to [Cu(D-A)(L-BH)] than to [Cu(L-A)(L-BH)], further substantiating that the *meso* complexes were preferentially obtained as crystals under the conditions employed.

It is interesting to note that, whereas X-ray structure analyses[49,50] have revealed that bis(amino acidato)copper(II)

Table 15. Yields and Optical Purities of the D-Enantiomers of Acidic Amino Acids Isolated *via* the Formation of the Ternary Complexes, Cu(A)(L-BH)

Ligand		Cu(A)(L-BH) isolated		AH_2 isolated	
AH_2	BH_2	Yield(%)[a]	Optical purity(%)[b]	Yield(%)[c]	Optical purity(%)[d]
DL-Asp	L-Arg	17	93	12	89
	L-Lys	28	79	11	89
	L-Orn	28	50	14	44
DL-Glu	L-Arg	61	42	53	33
	L-Lys	18	75	14	70
	L-Orn	21	39	12	35

a) Yield of the isolated complex based on the amount of copper(II) used.

b) Optical purity of AH_2 estimated from the calibration curves shown in Figure 20.

c) Yield of isolated AH_2 based on the half amount of DL-AH_2 used.

d) Estimated from the specific rotation, $[\alpha]_{589}^{20}$ (in 3 M HCl; c=1).

Table 16. Yields and Optical Purities of the D-Enantiomers of Basic Amino Acids Isolated *via* the Formation of the Ternary Complexes, Cu(L-A)(BH)

Ligand		Cu(L-A)(BH) isolated		BH_2 isolated	
BH_2	AH_2	Yield(%)[a]	Optical purity(%)[b]	Yield(%)[c]	Optical purity(%)[d]
DL-Arg	L-Glu	36	46	14	41
	L-Asp	63	42	30	38
DL-Lys	L-Glu	18	56	7	50
	L-Asp	39	16	21	11
DL-Orn	L-Glu	36	12	19	17
	L-Asp	34	42	29	36

a) Yield of the isolated complex based on the amount of copper(II) used.

b) Optical purity of BH_2 estimated from the calibration curves shown in Figure 21.

c) Yield of isolated BH_2 based on two-thirds of the amount of DL-BH_2 used.

d) Estimated from the specific rotation, $[\alpha]_{589}^{20}$ (in 3 M HCl; c=1).

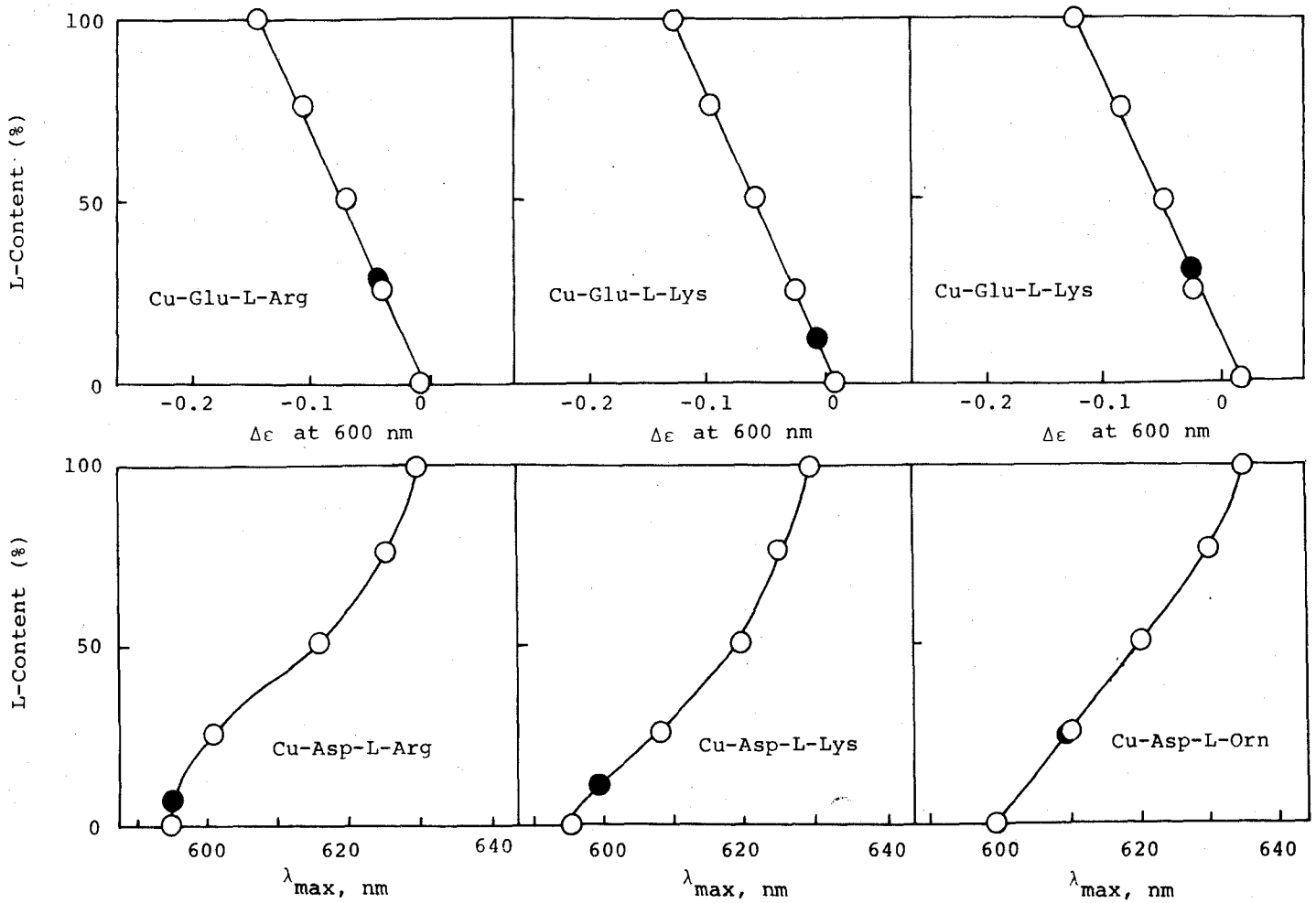

Figure 20. Determination of the Optical Purities of Acidic Amino Acids Incorporated into Cu(A)(L-BH) (●) from the Calibration Curves Based on the CD Spectra of the Standard Samples(○).

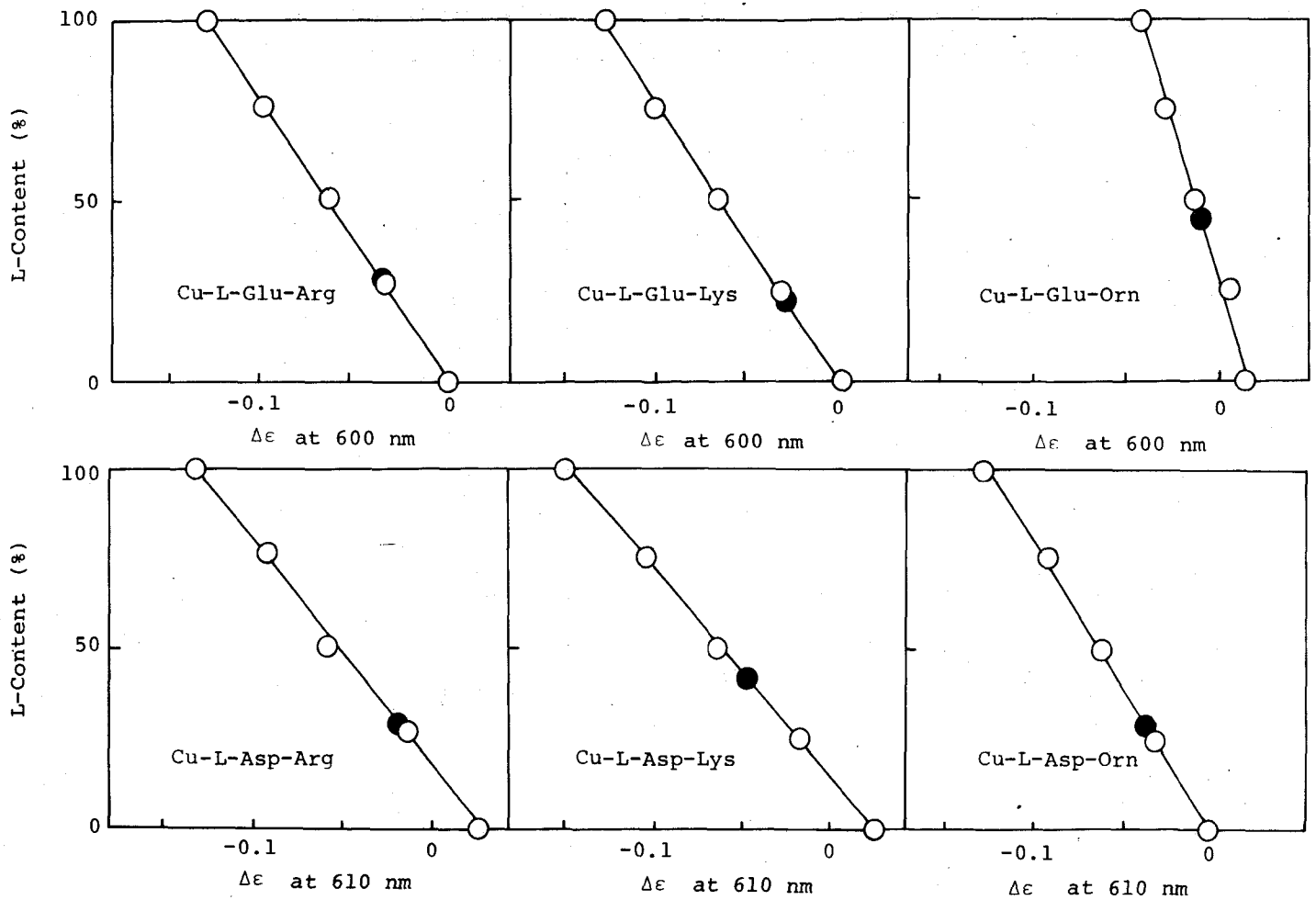

Figure 21. Determination of the Optical Purities of Basic Amino Acids Incorporated into Cu(L-A)(BH) (●) from the Calibration Curves Based on the CD Spectra of the Standard Samples(○).

complexes assume a *cis* structure while others assume a *trans* one, every possible combination of AH_2 and BH_2 gives as the main product a *meso* complex probably with a *cis* structure.

Factors Affecting the Optical Resolution of Asp, Glu, Arg, Lys and Orn

A potentiometric study is a reliable source of information about the species distribution and hence the stereoselectivity in solution. However, it showed the enantiomeric pairs of the ternary complexes give almost identical stability constants at the ionic strength of 0.1(KNO_3) (Table 9) and almost identical titration curves at a lower ionic strength or in aqueous ethanol. Accordingly, it may be unrealistic to expect large stability differences between the enantiomeric species present in the reaction media. No significant difference was observed between the CD spectra in the d-d region of 1 : 1 : 1 and 1 : 2 : 1 mixtures of Cu(II), DL-AH_2, and L-BH_2, which indicates that there exists no remarkable preference of *cis*-[Cu(D-A)-(L-BH)] over *trans*-[Cu(L-A)(L-BH)] in solution and that the two isomers are approximately equally present. In contrast to this, the ternary complex isolated from a 1 : 1 : 1 mixture of Cu(II), L-Glu, and DL-Orn incorporated the D-enantiomer with 17% enantiomeric excess. These findings suggest that the solubility factor rather than the stability factor plays as important role in the optical resolution *via* complex formation.

In conclusion, the electrostatic interactions between the

ligands within a complex molecule serve as an essential force fixing the structure of the complex in a particular configuration.

Optical Resolution of Histidine

By using the solubility difference between the diastereomers Cu(L-asn)(L-his) and Cu(D-asn)(L-his), DL-His was resolved into enantiomers *via* isolation of [Cu(L-asn)(his)] from a neutral solution of Cu^{2+}, DL-His, and L-Asn in water or aqueous methanol. Depending on the conditions used for crystallization, the mixture gave the analytically pure complexes. From the CD calibration curve(Figure 22) and the specific rotations of His isolated from the complexes(Table 16), the complex isolated from aqueous methanol is shown to have incorporated the L-enantiomer almost exclusively with 98% optical purity. Since solvents with low polarity, such as aqueous methanol, favors hydrogen bond formation, the fact strongly suggests the importance of intramolecular ligand-ligand interactions between the polar side groups that would assist the complex formation and fix the ligand in a suitable conformation and configuration. Such an explanation is quite consistent with the structure of [Cu(L-asn)(L-his)](structure 4 in Chapter 4), which indicates that the conformations of the side chain of Asn is favorable for the intramolecular bondings and that the bondings have been maintained untill they are broken to form the intermolecular hydrogen bonds necessary for the crystal propagation.

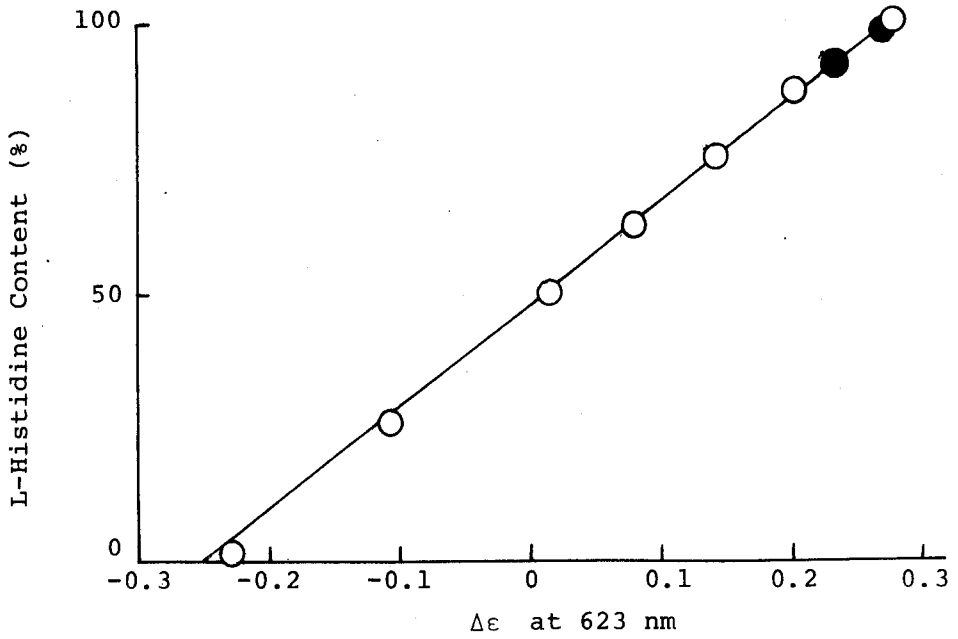

Figure 22. Determination of the Optical Purities of Histidine Incorporated into Cu(L-asn)(his)(●) from the Calibration Curve Based on the CD Spectra of the Standard Samples(○) at I=0.1(KNO_3).

Table 16. Optical Resolution of DL-Histidine *via* Complex Formation

System	Complex isolated	Yield(%)[a]	Optical purity(%)[b]	
			Complex isolated[c]	His isolated[d]
Cu(II)-L-Asn-DL-His (1:1:1.5)	[Cu(L-asn)(his)]·0.25H_2O	25	98	98
	[Cu(L-asn)(his)]·3.5H_2O	24	84	88

a) The yield of the isolated complex based on the amount of copper(II) used.

b) The optical purity of L-histidine incorporated.

c) Estimated by the CD calibration curve.

d) Calculated from the specific rotations $[\alpha]_{589}^{20}$. The samples contained a small amount(*ca.* 4%) of inorganic impurities, which were not removed by recrystallization.

Stereoselective incorporation of enantiomers of histidine into the ternary complexes containing L-Gln, L-Thr or L-Ser was not feasible, either because analytically pure ternary complexes were not isolated or because ternary complexes containing equal amounts of L- and D-histidine were isolated.

Chapter 6

Concluding Remarks

With a view to mimicking the specificity exhibited by enzymes and getting insights into biological reaction mechanism, synthetic, spectroscopic and solution equilibrium studies have been made on the ternary copper(II) complexes containing two optically active α-amino acids with charged groups in their side chains. For all combinations of ligands AH_2 and BH_2, where AH_2 refers to aspartic or glutamic acid and BH_2 to arginine, lysine or ornithine, the mixed complexes [Cu-(L-A)(L-BH)] and [Cu(D-A)(L-BH)] were isolated as crystals. Infrared spectra of the isolated complexes suggested the presence of the geometric isomers. By comparing the observed circular dichroism spectra of the ternary systems with the spectra estimated from those of the corresponding binary systems, the magnitude enhancements were detected for all the Cu-L-AH_2-L-BH_2 systems, whereas no such enhancements were observed when one of the ligands was L-alanine or L-valine. The spectral behaviors as well as the properties of the solid complexes isolated have been interpreted as indicative of the existence of the intramolecular electrostatic bondings between the oppositely charged groups in the side chains of the ligands

which were further confirmed by the remarkable influence of inorganic salts and polarity of solvent on the CD spectra. Moreover, stability constants of the mixed ligand complexes were determined by analyzing the pH titration data with a computer program SCOGS, and it was recognized that the ternary species are the predominant species in the neutral-alkaline pH range. Optical resolution of DL-aspartic and DL-glutamic acids has been performed *via* formations of ternary copper(II) complexes composed of DL-AH_2 and L-BH_2 or L-AH_2 and DL-BH_2. For all combinations of ligands, the *meso* complex [Cu(L-A)-(D-BH)] and [Cu(D-A)(L-BH)] with *cis* configuration were preferentially isolated. The optical purities of the resolved amino acids were as high as 90 and 70% for aspartic and glutamic acid, respectively, and 40-50% for the basic amino acids, arginine, lysine and ornithine. These findings show that electrostatic interactions within complex molecules serve as a novel effective driving force for the ternary complex formation, simultaneously exhibiting stereoselectivity. The mentioned complexes are regarded as models for the enzyme-metal-substrate complex, because, through the electrostatic interaction between the charged group of an enzyme and that of a substrate, the enzyme specifically selects its substrate and fixes it at the proper position for the reaction.

Ultracentrifugation and ultrafiltration studies[26-32] have suggested the presence of low molecular weight copper carriers in blood serum, where L-histidine-containing ternary

copper(II) complexes with L-threonine, L-asparagine, L-glutamine have been detected and have received special attention these ten years. However, X-ray crystal analysis of [Cu-(L-his)(L-thr)] and some solution equilibrium studies have not revealed why some L-histidine-containing ternary complexes appear to be particularly important as copper carriers under physiological conditions. L-Asn, L-Gln and L-Thr, which are found to be involved in such complexes, all carry potent hydrogen bond-forming groups in the side chains, and these possibly interact directly or indirectly with the carboxylate group of histidine coordinated at the apical position. In order to substantiate that these interactions are driving forces for formation of those histidine-containing ternary comlexes, synthetic, spectroscopic and solution equilibrium studies have been performed.

Although the X-ray crystal structure analyses of the two modifications of [Cu(L-asn)(L-his)] implicated the importance of intermolecular hydrogen bondings for the crystallization of the Asn-, Gln-, Thr-,and Ser-containing ternary crystals with histidine all of which were isolated as crystals, the side chain conformation of one of the modifications and the result of optical resolution of histidine *via* complex formation strongly suggests the importance of the intramolecular hydrogen bonding, which can ultimately lead to stereoselective formation of the ternary complexes. On the other hand, CD spectral approach indicated the probable existence of the

intramolecular interaction, although it may not be a strong driving force for the ternary complex formation at 25°C and ionic strength of 0.1(KNO_3) as revealed by the determination of stability constants. The findings indicate that the proposal made from the ultracentrifugation and ultrafiltration studies[26-32] might need further evidence supporting the importance of some histidine-containing ternary copper(II) complexes as copper carriers in blood serum.

The ligand-ligand interactions are concluded to be novel and effective driving forces for the ternary complex formations and to give rise to stereoselectivities and specificities, which are in line with those frequently observed in biological systems. Moreover, the interactions are expected to affect many catalytic processes, which will offer a variety of further subjects to be investigated. A recent report[51] on the acceleration of the hydrolysis of peptides around the central metal ion by an intramolecular hydrogen bonding may illustrate an example.

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1) Mixed Ligand Copper(II) Complexes of *α*-Amino Acids with Ligand-Ligand Interactions.
T. Sakurai, O. Yamauchi and A. Nakahara, *Bull. Chem. Soc. Jpn.*, 49, 169(1976).

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