Mixed Ligand Copper(II) Complexes of Amino Acids and Related Compounds with Possible Ligand-Ligand Interactions

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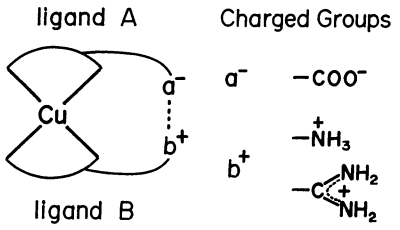
Ternary systems composed of copper(II) and two ligands, each with an oppositely charged uncoordinated group in the side chain, have been investigated by synthetic and spectral methods. The absorption and circular dichroism(CD) spectra in the d-d region were measured at various pH values for the binary and ternary systems, $Cu(B)_2$ and Cu(A)(B), where A refers to ethylenediamine-*N*-monoacetic acid(EDMA), glycylglycine(Gly·Gly), glycyl-β-alanine(Gly·β-Ala), ethylenediamine(en), or glycine(Gly), and B to D- and L-arginine (D- and L-Arg), L-ornithine(L-Orn), L-lysine(L-Lys), L-alanine(L-Ala), or L-valine(L-Val), and the following complexes containing ligands with polar side chains were isolated as crystals: [Cu(EDMA)(L-Arg)]$ClO_4 \cdot H_2O$; [Cu(EDMA)(L-Orn)]-$ClO_4 \cdot 1/2H_2O$; [Cu(EDMA)(L-Lys)]ClO_4; [Cu(Gly·Gly)(L-Arg)]$\cdot 3/2H_2O$. From comparison of the CD magnitudes expressed in terms of the relative magnitude which assumes half the value for $Cu(B)_2$ as unity, the magnitude enhancements observed for the systems Cu(EDMA)(D- and L-Arg), Cu(EDMA)(L-Orn), Cu(EDMA)-(L-Lys), and Cu(Gly·β-Ala)(D- and L-Arg) in water and in 50% aqueous ethanol have been inferred to be due to the intramolecular electrostatic interactions between the charged groups in the side chains of ligands A and B.

Transition metal ions in the biological systems are most probably in the forms of various mixed complexes. Thus, the enzyme–metal–substrate complex, formed as an intermediate in the enzymatic reaction in the presence of a metal ion, is a kind of mixed ligand complex, and the reaction itself is regarded as a ligand-ligand interaction assisted by the cooperative action of the central metal ion. A well known example is the carboxypeptidase A–peptide complex whose crystal structure has been disclosed by X-ray analysis.[1] What appeared most interesting to us is that it has electrostatic or hydrogen bondings between the guanidinium group of arginine 145 of the enzyme and the carboxylate terminus of its substrate. Since such an interaction serves as a driving force for the enzyme-substrate complex formation, we considered it to be of fundamental importance to work out reasonable model systems based on the carboxypeptidase A–peptide complex and investigate, as a chemical counterpart of the enzymatic reactions, the possibilities of ligand-ligand interactions within mixed ligand complex molecules.

Studies have been reported for the stereoselective effects of the interactions between coordinated ligands in various cobalt(III) complexes, where, for example, the uncoordinated carboxylate group around the central atom interacts with the adjacent coordinated amino group through hydrogen bonding. Such interactions have been concluded to give rise to stereoselectivity in the formation of the diastereoisomers.[2–5] Wellman and Wong[6] observed favored formation of the L-histidinol-containing mixed ligand complexes of copper(II), whose circular dichroism(CD) spectra suggested the interaction between the imidazole group of L-histidinol and the amino group of the other ligand. Angelici *et al.*[7,8] reported the stereoselectivity exhibited by (*N*-carboxymethyl-L-valinato)copper(II) toward optically active amino acids and ascribed it to the interference between the amine hydrogens of the two ligands in the resulting complexes. However, mixed ligand systems involving explicit electrostatic interactions within a molecule have not been reported yet.

As an approach to the biological systems, we studied the properties of the mixed ligand copper(II) complexes of the type shown in Scheme 1 in expectation of intramolecular electrostatic ligand-ligand interactions that would occur between the oppositely charged, uncoordinated groups of the side chains of ligands A and B.



Scheme 1.

We selected for this purpose such pairs of A and B as would exhibit the mentioned interactions, as well as the pairs without possible interactions, and investigated isolation and absorption and CD spectral properties of the copper(II) complexes of type Cu(A)-(B), which are to be described in the following sections. For convenience, ethylenediamine-*N*-monoacetic acid (EDMA), glycylglycine(Gly·Gly), glycyl-β-alanine (Gly·β-Ala), ethylenediamine(en), and glycine(Gly) will be referred to as A ligands, and D- and L-arginine (D- and L-Arg), L-ornithine(L-Orn), L-lysine(L-Lys), L-alanine(L-Ala), and L-valine(L-Val) as B ligands.

Experimental

Materials. L-Arginine, L-ornithine hydrochloride, L-lysine dihydrochloride, and L-alanine were purchased from Nakarai Chemicals, Ltd., and L-valine and glycine from Wako Pure Chemical Industries, Ltd. D-Arginine hydrochloride was obtained from Fluka AG. Glycylglycine was obtained from Protein Research Foundation, Osaka, and glycyl-β-alanine was prepared according to the literature.[9] The dihydrochloride of EDMA was prepared by the method of Fujii *et al.*,[10] and the purity was checked by elemental analysis and potentiometric titration.

All the chemicals used were of reagent grade or of highest grade available.

Synthesis of Mixed Ligand Copper(II) Complexes. [*Cu(EDMA)(L-Arg)*]$ClO_4 \cdot H_2O$: EDMA dihydrochloride hydrate (1.05 g, 5 mmol), L-arginine (0.87 g, 5 mmol), and copper(II) perchlorate hexahydrate (1.85 g, 5 mmol) were mixed in water, and the pH of the resulting solution was adjusted to 8 with aqueous sodium hydroxide. The chloride ions were removed from the reaction mixture by passing it through a column of Amberlite IRA-400 in perchlorate form. The blue oily residue obtained after concentration *in vacuo* was mixed with methanol and kept at room temperature for a few days, when it gradually crystallized. Recrystallization from aqueous methanol–ethanol and drying *in vacuo* over silica gel gave deep blue crystals. Found: C, 25.40; H, 5.35; N, 17.80%. Calcd for $C_{10}H_{23}N_6O_8ClCu \cdot H_2O$: C, 25.43; H, 5.34; N, 17.79%.

[*Cu(EDMA)(L-Orn)*]$ClO_4 \cdot 1/2H_2O$: A neutralized solution (pH 7) of EDMA dihydrochloride hydrate (1.05 g, 5 mmol), L-ornithine hydrochloride (0.84 g, 5 mmol), and copper(II) chloride dihydrate (0.85 g, 5 mmol) in water was treated in the manner described above to give deep blue crystals. Found: C, 25.84; H, 5.22; N, 12.88%. Calcd for $C_9H_{21}N_4O_8ClCu \cdot 1/2H_2O$: C, 25.87; H, 5.22; N, 13.29%.

[*Cu(EDMA)(L-Lys)*]ClO_4: This was prepared in a similar manner from EDMA dihydrochloride hydrate (1.05 g, 5 mmol), L-lysine dihydrochloride (1.10 g, 5 mmol), and copper(II) chloride dihydrate (0.85 g, 5 mmol). The deep blue crystals were recrystallized from methanol–water and dried *in vacuo* over P_4O_{10} at 60 °C. Found: C, 28.18; H, 5.56; N, 12.77%. Calcd for $C_{10}H_{23}N_4O_8ClCu$: C, 28.17; H, 5.44; N, 13.14%.

[*Cu(Gly·Gly)(L-Arg)*]$\cdot 3/2H_2O$: An aqueous solution containing Gly·Gly (1.3 g, 10 mmol), L-arginine (1.7 g, 10 mmol), and copper(II) perchlorate hexahydrate (3.7 g, 10 mmol) was made alkaline (pH 10) with aqueous sodium hydroxide, stirred for 1 hr at room temperature, and filtered. Ethanol was added to the filtrate to give deep blue crystals, which were recrystallized from aqueous methanol–ethanol. Found: C, 30.41; H, 5.88; N, 21.28%. Calcd for $C_{10}H_{20}N_6O_5Cu \cdot 3/2H_2O$: C, 30.57; H, 5.60; N, 21.42%.

Measurements of Spectra. Absorption spectra were recorded on a Union Giken SM-401 High-Sensitivity recording spectrophotometer and CD spectra on a JASCO MOE-1 spectropolarimeter. The spectra were measured for most of the systems with Cu(II): A: B ratios of 1: 2: 0, 1: 0: 2, and 1: 1: 1 at various pH values in the ranges 350—750 nm (absorption spectra) and 350—800 nm (CD spectra). The measurements were made at a constant copper(II) concentration of 5.0×10^{-3} M in four different media: (i) water (ionic strength (μ) not adjusted (μ=variable)); (ii) water (μ=0.1 ($NaClO_4$)); (iii) water (μ=0.5 ($NaClO_4$)); (iv) 50% aqueous ethanol. The pH values of the solutions were roughly adjusted with aqueous sodium hydroxide and dilute perchloric acid and finally determined immediately after the spectral measurements. The values described are meter readings with no corrections for difference in the medium.

The above measurements were made at room temperature.

Results and Discussion

Synthesis of the Mixed Ligand Complexes. Out of the mixed ligand systems investigated, [Cu(EDMA)(L-Arg)]ClO_4, [Cu(EDMA)(L-Orn)]ClO_4, [Cu(EDMA)-(L-Lys)]ClO_4, and [Cu(Gly·Gly)(L-Arg)] have been isolated as crystals under the conditions employed. The pH of the reaction mixture was so adjusted as to keep the most basic group of the α-amino acid protonated.

Table 1. Absorption spectral data for binary systems $Cu(A)_2$ and $Cu(B)_2$ at various pH values (μ=0.1($NaClO_4$))

Ligand	pH	λ_{max} (nm)	ε
EDMA	4.1	668	72
	7.8	616	59
	10.8	616	59
Gly·Gly	4.5	651	43
	7.2	635	83
	8.8	624	83
	10.3	631	79
en	4.5	690	38
	7.1	546	62
	9.2	546	62
L-Arg	4.6	684	31
	7.0	622	54
	9.6	620	57
L-Orn	4.7	675	34
	6.8	623	53
	8.3	620	57
	10.1	632	64
	11.1	636	64
L-Lys	4.7	681	32
	7.0	619	54
	8.1	618	57
	10.1	618	58
	11.1	621	58
L-Ala	4.6	704	30
	7.1	622	52
	9.8	619	56
L-Val	4.7	687	32
	7.2	617	57
	10.0	616	58

The compositions of the isolated complexes indicate that each complex involves a ligand with a negatively charged uncoordinated group and a ligand with a positively charged one. The behavior of these particular systems may be correlated with the stereoselectivity reported for cobalt(III) complexes.[2-5] In the present cases, ligand-ligand interactions, which should be electrostatic in nature, are reasonably expected to occur between the charged groups of the side chains (Scheme 1).

Absorption Spectra. The absorption spectra in the d-d region were measured in water (μ=variable, 0.1, and 0.5) and in 50% aqueous ethanol at various pH values. The shifts of the absorption maxima of the binary and ternary systems due to the increase of ionic strength were small (*ca.* 2—4 nm toward shorter wavelength), and their intensities remained virtually constant at different ionic strengths except that they slightly increased in 50% aqueous ethanol. Table 1 shows the absorption spectral data for various binary systems at μ=0.1. The data for the ternary Cu(A)(B) systems at μ=variable are summarized in Table 2.

The absorption maxima of the ternary systems containing EDMA, Gly·Gly, Gly·β-Ala, or Gly as ligand A fall in the region 610—640 nm at pH>9 where Cu(A)(B) is expected to be the major complex species

TABLE 2. ABSORPTION SPECTRAL DATA FOR TERNARY SYSTEMS AT VARIOUS pH VALUES (μ=variable)

Ligand B	Ligand A: EDMA pH	EDMA λ_{max} (nm)	EDMA ε	Gly·Gly pH	Gly·Gly λ_{max} (nm)	Gly·Gly ε	Gly·β-Ala pH	Gly·β-Ala λ_{max} (nm)	Gly·β-Ala ε	en pH	en λ_{max} (nm)	en ε	Gly pH	Gly λ_{max} (nm)	Gly ε
L-Arg	7.2	633	63	7.1	638	72	7.9	628	66	7.2	583	55	7.0	630	48
	8.3	620	62	10.1	635	75	10.2	616	65	10.3	584	57	9.2	627	51
	9.6	620	61												
D-Arg	7.3	632	62	7.2	638	72	9.9	618	65	9.6	580	57	7.2	630	48
	9.8	620	62	10.2	636	74							10.0	627	51
L-Orn	7.0	640	62	7.5	635	72	7.1	629	65	7.0	583	54	7.2	628	48
	10.3	623	64	10.1	628	77	8.1	626	66	10.2	591	58	9.2	632	53
	11.0	627	65	11.5	639	73	10.4	618	69	11.2	594	58			
L-Lys	7.1	639	64	7.2	637	73	7.7	628	70	7.1	582	55	7.0	630	47
	9.0	619	63	8.9	626	75	8.9	620	67	8.9	580	57	9.9	628	50
	10.1	619	64	10.5	627	76	10.5	613	66	10.3	583	57			
	11.4	622	64	11.4	636	76									
L-Ala	7.1	645	63	7.0	640	73	10.1	619	67	7.0	584	54	7.3	630	47
	10.3	620	62	10.3	634	73				10.2	584	56	10.0	627	50
L-Val	7.1	640	64	7.0	638	73	7.7	627	67	7.0	583	56	7.0	629	48
	10.2	617	64	10.2	632	72	10.1	619	66	10.2	583	59	9.8	624	51

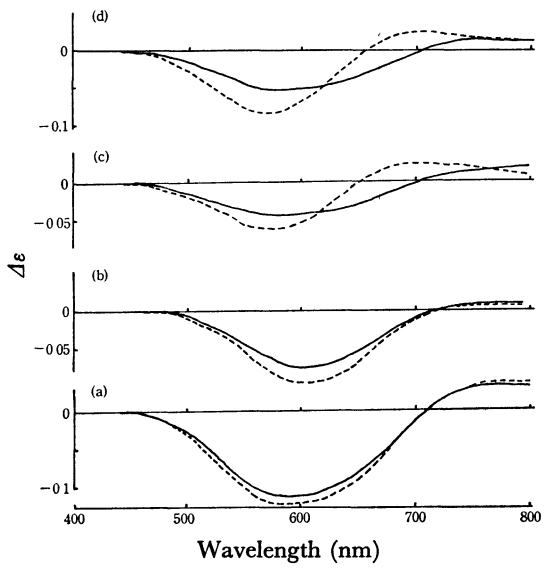


Fig. 1. Visible CD spectra of L-arginine-containing systems in water (μ=variable).
(a) Cu(L-Arg)$_2$: ——, pH 7.1; ----, pH 9.2
(b) Cu(EDMA)(L-Arg): ——, pH 7.2; ----, pH 8.3
(c) Cu(Gly·Gly)(L-Arg): ——, pH 7.1; ----, pH 10.1
(d) Cu(Gly·β-Ala)(L-Arg): ——, pH 7.9; ----, pH 10.2.

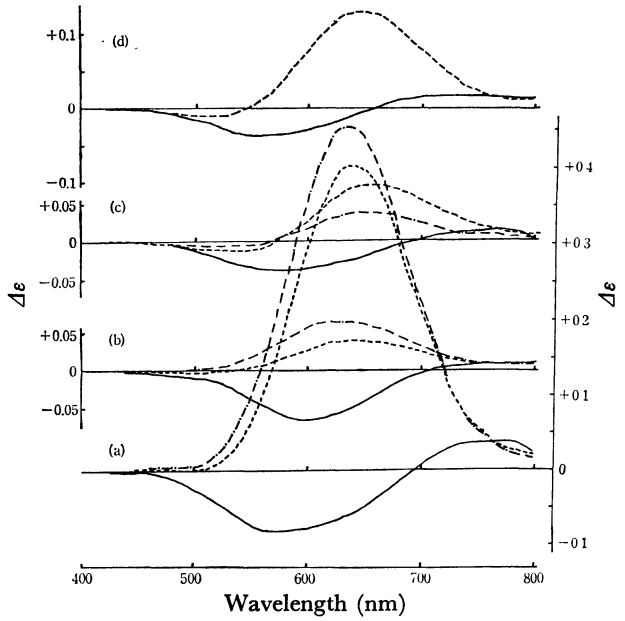


Fig. 2. Visible CD spectra of L-ornithine-containing systems in water (μ=variable).
(a) Cu(L-Orn)$_2$: ——, pH 7.1; ----, pH 9.9; -·-·-, pH 10.8
(b) Cu(EDMA)(L-Orn): ——, pH 7.0; ----, pH 10.3; -·-·-, pH 11.0
(c) Cu(Gly·Gly)(L-Orn): ——, pH 7.5; ----, pH 10.1; -·-·-, pH 11.5
(d) Cu(Gly·β-Ala)(L-Orn): ——, pH 8.1; ------, pH 10.4.

present. As we see from the data for Cu(A)(L-Orn) and Cu(A)(L-Lys), some of the spectra exhibit shifts to longer wavelengths at pH>10, probably due either to apical coordination by the δ- or ε-amino group or to formation of hydroxy complexes. This point will be referred to again in connection with the CD spectra.

CD Spectra. In the d-d spectral region, the binary and ternary systems containing optically active amino acids exhibited CD extrema due to the vicinal effect of the asymmetric carbon. Figs. 1 and 2 illustrate the spectra for the systems involving L-arginine and L-ornithine, respectively. The spectral data in water (μ=variable) at selected pH are listed in Table 3. Usually a negative extremum, which is shifted to longer wavelengths at μ=0.1 and 0.5, is observed in the region 570—610 nm with a weak positive peak at >700 nm.

TABLE 3. CD SPECTRAL DATA FOR BINARY AND TERNARY SYSTEMS (μ=variable)

Ligand B	Binary system Cu(B)$_2$			Ternary system Cu(EDMA)(B)			Ternary system Cu(Gly·Gly)(B)		
	pH	λ_{max} (nm)	$\Delta\varepsilon$	pH	λ_{max} (nm)	$\Delta\varepsilon$	pH	λ_{max} (nm)	$\Delta\varepsilon$
L-Arg[a]	7.1	598	−0.11	7.2	606	−0.08	7.1	588	−0.04
		≃765	+0.03		>760	+0.01		>760	+0.01
	9.2	598	−0.12	8.3	606	−0.10	10.1	574	−0.06
		≃765	+0.03		>760	+0.01		700	+0.02
D-Arg	6.9	598	+0.12	7.3	610	+0.08	7.2	590	+0.04
		≃765	−0.03		>760	−0.02		≃755	−0.02
	10.1	598	+0.11	9.8	610	+0.10	10.2	574	+0.06
		≃765	−0.03		>760	−0.02		700	−0.02
L-Orn	7.1	575	−0.08	7.0	600	−0.07	7.5	570	−0.04
		≃760	+0.04		>740	+0.01		>760	+0.01
	9.9	632	+0.40	10.3	643	+0.04	10.1	654	+0.07
	10.8	630	+0.45	11.0	630	+0.06	11.5	650	+0.04
L-Lys	7.1	600	−0.11	7.1	607	−0.06	7.2	588	−0.05
		>750	+0.03		>750	+0.01		>780	+0.02
	9.4	600	−0.13	9.0	607	−0.09	8.9	578	−0.06
		>750	+0.03		>750	+0.01		740	+0.02
	10.8	600	−0.14	11.4	607	−0.09	11.4	578	−0.03
		>750	+0.03		>750	+0.01		≃740	+0.01
L-Ala	7.0	625	−0.07	7.1	610	−0.03	7.0	615	−0.03
		>760	+0.01		>760	+0.01			
	10.1	625	−0.08	10.3	610	−0.04	10.3	590	−0.05
		>760	+0.01		>760	+0.01		≃740	+0.02
L-Val	7.3	596	−0.28	7.1	612	−0.08	7.0	595	−0.10
		≃780	+0.05		≃780	+0.02		>760	+0.02
	9.8	596	−0.29	10.2	612	−0.12	10.2	587	−0.12
		≃780	+0.05		≃780	+0.03		752	+0.04

Ligand B	Ternary system Cu(Gly·β-Ala)(B)			Ternary system Cu(en)(B)			Ternary system Cu(Gly)(B)		
	pH	λ_{max} (nm)	$\Delta\varepsilon$	pH	λ_{max} (nm)	$\Delta\varepsilon$	pH	λ_{max} (nm)	$\Delta\varepsilon$
L-Arg[a]	7.9	576	−0.05	7.2	596	−0.05	7.0	604	−0.06
		>750	+0.02		≃750	+0.01		>760	+0.02
	10.2	570	−0.08	10.3	596	−0.05	9.2	604	−0.07
		710	+0.02		≃750	+0.01		>760	+0.02
D-Arg	9.9	570	+0.08	9.6	595	+0.05	7.2	604	+0.06
		710	−0.02		≃750	−0.01		>760	−0.02
							10.0	604	+0.06
								>760	−0.02
L-Orn	7.1	580	−0.04	7.0	596	−0.04	7.2	600	−0.04
		>740	+0.01		>740	+0.01		>740	+0.01
	8.1	556	−0.04	10.2	616	+0.14	9.2	633	+0.11
		>720	+0.02	11.2	610	+0.15			
	10.4	503	−0.01						
		644	+0.13						
L-Lys	7.7	602	−0.05	7.1	595	−0.05	7.0	607	−0.06
		>740	+0.02		≃750	+0.01		>760	+0.02
	8.9	574	−0.06	8.9	595	−0.06	9.9	607	−0.07
		≃750	+0.02		≃750	+0.01		>760	+0.02
	10.5	573	−0.06	10.3	595	−0.06			
		710	+0.02		≃750	+0.01			
L-Ala	10.1	589	−0.05	7.0	614	−0.03	7.3	630	−0.04
		≃740	+0.01	10.2	614	−0.04	10.0	630	−0.04
L-Val	7.7	595	−0.12	7.0	580	−0.12	7.0	604	−0.13
		≃770	+0.02		≃770	+0.02		>760	+0.03
	10.2	580	−0.12	10.2	580	−0.13	9.8	604	−0.14
		≃750	+0.03		≃770	+0.02		>760	+0.03

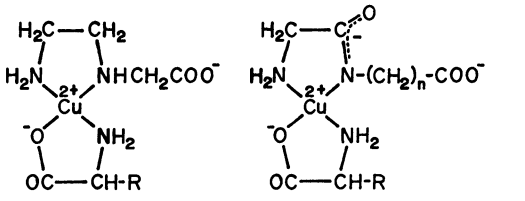
a) The data (pH, λ_{max} (nm), $\Delta\varepsilon$) for Cu(EDMA)(L-Arg) at higher concentrations are as follows: (i) at 0.05 M, 8.2, 603, −0.11; (ii) at 0.1 M, 9.0, 604, −0.11.

The magnitudes of the negative peaks are small in acid solution (pH 3—5) and increase with pH up to their maximum at pH 9—10, which indicates the chelation by the amino acid is almost complete at this pH. That the CD spectra observed for the ternary systems are due primarily to Cu(A)(B) and not to the binary $Cu(B)_2$ is substantiated by the wavelengths of their extrema which are significantly different from those of the binary ones. Except for the L-ornithine series, the systems Cu(EDMA)(B), Cu(en)(B), and Cu(Gly)(B) at pH>7 have their peaks at 600—612, 580—616, and 600—630 nm, respectively, and the wavelengths do not shift greatly with pH. On the other hand, the systems with Gly·Gly or Gly·β-Ala as ligand A have negative bands around 570—600 nm, which are shifted to shorter wavelengths with the increase of pH. The behavior may be due to the deprotonation of the peptide NH group and the concomitant change of the donor atom from oxygen to nitrogen.

A sign inversion of the main peak was observed for $Cu(L\text{-Orn})_2$ and every Cu(A)(L-Orn) system studied, and there appeared a positive peak in the region 610—650 nm at pH 9—10. The same spectral changes have been reported by Wellman *et al.*[11] and Wilson *et al.*[12] for the systems Cu(Gly)(L-Orn)[11,12] and Cu(en)-(L-Orn).[12] It was concluded from their studies that the change is due to apical coordination by the δ-amino group of L-ornithine in these systems. The present observation that the sign inversion coincides with the acid dissociation of the δ-amino group (pK_a=10.67[13]) supports their conclusion, and apical coordination by this group is inferred to occur in all the L-ornithine-containing systems studied. The shifts of the absorption maxima to longer wavelengths at high pH correspond with those observed for copper(II)–ammine complexes.[14] Apical coordination by the ε-amino group of L-lysine, if at all, seems to be negligible, because the absorption and CD spectral changes exhibited at pH 8—10 are small.

CD Magnitude and Intramolecular Ligand-Ligand Interaction. Of utmost interest is the fact that the CD magnitudes shown in Table 3 have a distinct trend which seems to reflect the differences in the environments where the amino acids are located. In order to make a clearer comparison of the magnitudes of the negative extrema in the region 570—620 nm, we tentatively expressed a series of magnitudes related to an amino acid in terms of the relative magnitude which assumes half the magnitude ($\Delta\varepsilon/2$) of the binary complex of that amino acid as unity. Table 4 displays the relative magnitudes calculated in this way for various systems in water (μ=variable) at pH as indicated. The values for L-ornithine series were calculated at pH 7 where its apical coordination does not take place appreciably. It is now apparent that the systems Cu(EDMA)(L-Arg), Cu(EDMA)(L-Orn), Cu(EDMA)(L-Lys), and Cu(Gly·β-Ala)(L-Arg) have significantly larger relative magnitudes than unity, while the others have values close to 1.

Although attempts have been made to interpret the CD spectral patterns of copper(II) complexes in the d-d region and to correlate them with the structures around copper(II),[15-22] it is still necessary to determine experimentally whether or not the relative CD magnitudes as defined above can be compared between different systems or, in other words, whether the additivity of the CD magnitude holds in complexes with differentcombinations of ligands. Tsangaris and Martin[23] reported the magnitude additivity observed for copper-(II)–dipeptide complexes. In the present studies, the donor atoms of A ligands are two nitrogens(2N) or a nitrogen and an oxygen(1N1O), and simple additivity may not hold owing to the differences in the environments. However, we see from Table 4 that the relative magnitudes observed for the Cu(en)(B) and Cu(Gly)-(B) series, where the donor atoms are 2 N and 1N1O, respectively, are 0.8—1.1 irrespective of B ligands.[24] Also, the ternary systems containing L-alanine or L-valine as B have magnitudes of 0.8—1.2 for all combinations with A. These results clearly indicate that the relative CD magnitude is approximately equal to 1 and accordingly additive unless both ligand A and ligand B have respectively a negatively charged and a positively charged group in their side chain. In addition, we may infer from this that the mode of coordination of the simple α-amino acids in the ternary systems studied is the same as in the binary systems. As α-amino acids are effective bidentate ligands, the carboxylate group of EDMA, Gly·Gly, or Gly·β-Ala are probably dis-



Cu(EDMA)(B) Ia

Cu(Gly·Gly)(B) (n=1)
Cu(Gly·β-Ala)(B) (n=2)
Ib

Table 4. Relative CD magnitudes in water (μ=variable)[a]

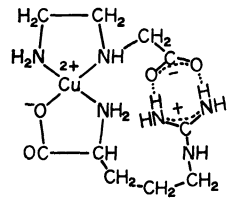
Ligand B	$Cu(B)_2$	Cu(EDMA)(B)	Cu(Gly·Gly)(B)	Cu(Gly·β-Ala)(B)	Cu(en)(B)	Cu(Gly)(B)
L-Arg	1.0(9.2)[b]	1.6(9.6)	1.0(10.1)	1.4(10.2)	0.8(10.3)	1.1(9.2)
D-Arg	1.0(10.1)	1.8(9.8)	1.1(10.1)	1.5(9.9)	1.0(9.6)	1.0(10.0)
L-Orn	1.0(7.1)	1.6(7.0)	0.9(7.5)	1.0(7.1)	0.9(7.0)	1.0(7.2)
L-Lys	1.0(9.4)	1.5(9.0)	1.0(8.9)	0.9(8.9)	1.0(8.9)	1.0(9.9)
L-Ala	1.0(10.1)	0.9(10.3)	1.1(10.3)	1.2(10.1)	1.0(10.1)	1.1(10.0)
L-Val	1.0(9.8)	0.8(10.2)	0.8(10.2)	0.8(10.2)	0.9(10.2)	1.0(9.8)

a) Relative CD magnitude is the magnitude relative to $\Delta\varepsilon/2$ of $Cu(B)_2$ as unity. b) Numerals in parentheses refer to the pH values where the comparison was made.

placed from the tetragonal plane upon formation of Cu(A)(B) (**1a** and **1b**).

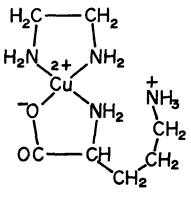
Considering that the magnitude enhancement is observed only for the combination of A and B with oppositely charged side chains and that the CD measurements have been performed at a copper(II) concentration of 5.0×10^{-3}M, we may take the enhancement as an indication of the presence of an intramolecular ligand-ligand interaction which adds to rigidity of the complex. The enhancement of the CD magnitude and the sign inversion have been reported for the Cu(bipyridyl)(L-amino acid) systems,[25] which are quite different in nature from the present ones.

Molecular models for Cu(EDMA)(L-Arg), Cu(EDMA)(L-Orn), and Cu(EDMA)(L-Lys) corroborate the favorable steric requirements for the assumed intramolecular hydrogen bonds as shown for Cu(EDMA)(L-Arg) (**2**). According to this structure, the imino nitrogen of EDMA can be asymmetric if it is fixed around copper(II) by hydrogen bonding. For the systems without charged pairs, such as Cu(en)(L-Orn) (**3**) and Cu(EDMA)(L-Ala) (**4**), no intramolecular hydrogen bonding is possible, and the observed CD magnitudes are expectedly close to **1**.



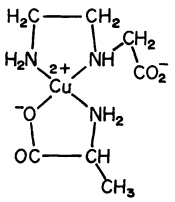
Cu(EDMA)(L-Arg)

2



Cu(en)(L-Orn)

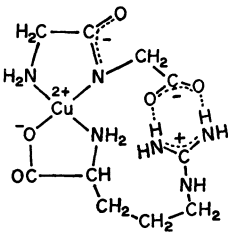
3



Cu(EDMA)(L-Ala)

4

As regards the Cu(Gly·Gly)(B) and Cu(Gly·β-Ala)(B) systems except Cu(Gly·β-Ala)(L-Arg), the relative magnitudes are 0.8—1.2 and apparently contradictory to the ligand-ligand bondings as are expected from the molecular models of the structures like **5**. Table 3 indicates that in the peptide series the positive peak found at >700 nm is shifted to shorter wavelength with the increase of pH, probably inducing the cancellation of the two Gaussian curves with signs opposite to each other. This may explain the unexpectedly small relative magnitudes. Space-filling models do not reveal explicitly the stereochemical difference between Cu(Gly·Gly)(L-Arg) and Cu(Gly·β-Ala)(L-Arg) of



Cu(Gly·Gly)(L-Arg)

5

which the latter has a significantly larger magnitude of 1.5 than that of the former.

From the CD spectral behavior of Cu(EDMA)(D-Arg), the CD patterns for the other D-amino acid series are expected to be the same as those observed for the corresponding L-series with the signs inverted.

If the assumed electrostatic ligand-lagand bonding as illustrated in **2** and **5** is real and has influence on the CD spectra, the relative magnitude should then be affected by the ionic strength and the polarity of the solvent. The results of the spectral measurements in water at μ=0.1 and 0.5 are compared with those at μ=variable in Table 5, which discloses that the enhanced values for Cu(EDMA)(L-Arg), Cu(EDMA)(L-Orn), and Cu(EDMA)(L-Lys) at μ=variable decrease to nearly normal values with the increase of ionic strength, while those for Cu(EDMA)(L-Ala), Cu(EDMA)(L-Val), and Cu(en)(B) remain unaffected. On the other hand, the data obtained in 50% aqueous ethanol indicate that the ionic strength-dependent values are further increased up to 2.1—2.7 by lowering the polarity of the solvent with ethanol. Interestingly, the CD patterns of Cu(EDMA)(L-Arg) at concentrations as high as 0.05 M (pH 8.2) and 0.1 M (pH 9.0) were the same as the pattern observed at 0.005 M (Table 3, footnote). This points to the fact that the molecular environment in the complex is not affected by the concentration of the solution in the range studied.

Taken together, the observations may provide evidence that there exists, at least in solution, an intramolecular electrostatic bonding in a certain pair of oppositely charged ligands constituting a mixed ligand copper(II) complex and that it affects the CD magnitude under favorable conditions.

We wish to acknowledge the helpful comments and

Table 5. Relative CD magnitudes in various media at pH 7—10

Ligand B	Cu(EDMA)(B)				Cu(Gly·Gly)(B)[a]				Cu(en)(B)			
	water, μ			50% aq. ethanol	water, μ			50% aq. ethanol	water, μ			50% aq. ethanol
	var.	0.1	0.5		var.	0.1	0.5		var.	0.1	0.5	
L-Arg	1.6	1.3	1.2	2.7	1.0	0.9	1.0	1.2	0.8	0.9	0.9	1.0
L-Orn	1.6	1.4	1.1	2.6	0.9	0.8	0.9	0.9	0.9	0.8	0.7	0.9
L-Lys	1.5	1.2	1.1	2.1	1.0	0.9	1.0	1.1	1.0	1.0	0.9	1.1
L-Ala	0.9	1.1	—[b]	—	1.1	1.1	—	—	1.0	1.0	—	—
L-Val	0.8	0.8	—	0.9	0.8	0.8	—	1.0	0.9	0.9	—	1.0

a) The values for Cu(Gly·β-Ala)(B) in 50% aqueous ethanol are as follows: L-Arg, 2.3; L-Orn, 0.9; L-Lys, 1.2.
b) Not measured.

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