Stereoselectivity in Mixed Ligand Copper(II) Complexes with Electrostatic Ligand-Ligand Interactions. Application to Optical Resolution of α-Amino Acids with a Charged Side Chain

Osamu YAMAUCHI, Takeshi SAKURAI, and Akitsugu NAKAHARA

Institute of Chemistry, College of General Education, Osaka University, Toyonaka, Osaka 560

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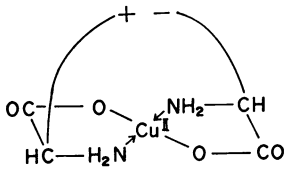
Optical resolution of DL-aspartic acid and DL-glutamic acid (abbreviated as DL-A) has been achieved *via* formation of ternary copper(II) complexes composed of a DL-A and L-arginine, L-lysine, or L-ornithine (L-B). For every pair of ligands DL-A and L-B, a neutralized solution containing $Cu(ClO_4)_2$, DL-A, and L-B in the molar ratio of 1 : 2 : 1 gave the mixed ligand complex abounding in [Cu(D-A)(L-B)] as crystals, from which incorporated A was isolated through a Dowex CCR-2 column after treating with H_2S. In a very similar manner D-enantiomer-rich B was obtained from DL-B by using an L-A. The optical purities of the resolved amino acids were as high as 90 and 70% for aspartic acid and glutamic acid, respectively, and 40—50% for the basic amino acids. The finding shows that the electrostatic ligand-ligand interactions in the mixed ligand complexes give rise to geometric isomerism around copper(II) and hence preferential formation and crystallization of the *meso* complexes, [Cu(L-A)(D-B)] and [Cu(D-A)(L-B)], probably with a *cis* configuration.

Stereoselectivity has been reported recently for kinetically labile transition metal complexes containing simple amino acids[1,2] and *N*-carboxymethyl[3] and *N*-benzyl[4] derivatives 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. In our previous studies electrostatic ligand-ligand interactions between the oppositely charged groups in the side chains of coordinated amino acids and related compounds have been inferred from the CD (circular dichroism) spectral magnitude enhancements in the d-d region, and interpreted as one of the driving forces leading to the formation of mixed ligand copper(II) complexes.[5,6] 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)(B), where A refers to the ionized form of aspartic acid (Asp) or glutamic acid (Glu) and B to the protonated form of arginine (Arg), lysine (Lys), or ornithine (Orn).[7] In fact,the infrared spectra of the isolated complexes indicated the existence of the geometric isomerism,[6a] and molecular models suggested a *trans* structure (**1**) for Cu(L-A)(L-B) and a *cis* structure (**2**) for Cu(D-A)(L-B).



Cu(L-A)(L-B)

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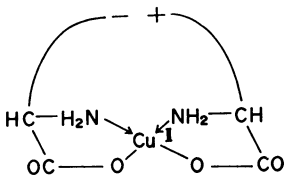

Cu(D-A)(L-B)

2

The stereoselectivity attributable to steric interactions between the side chains of the 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.[8] 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.

On the other hand, the stereoselectivity due to the electrostatic ligand-ligand interactions suggested the possibility of optical resolution of amino acids with a charged side chain, such as aspartic acid, glutamic acid, arginine, and lysine, which have been excluded from the resolution based on the bulkiness of the side chains. We reported in a previous communication[9] that racemic aspartic acid and glutamic acid could be resolved into enantiomers by using copper(II) and an optically active basic amino acid, L-B, and now we have investigated the stereoselectivity in detail to explore a novel approach to optical resolution of the enantiomers of the mentioned amino acids *via* formation of mixed ligand copper(II) complexes.

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. Their purity was checked by the specific rotations, $[\alpha]_{589}^{20}$ (in 3M 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). All the materials used were of reagent grade or of highest grade available.

Measurements. Absorption spectra of the complexes were measured with a Union Giken SM-401 High-Sensitivity recording spectrophotometer and CD spectra with a JASCO MOE-1 spectropolarimeter in a 1-cm or a 2-cm quartz cell. The spectral measurements were made in the range 400—800 nm at a constant copper(II) concentration of 5.0×10^{-3} M in water at room temperature. The pH values (7.5—8.5) of the solutions were roughly adjusted with aqueous sodium hydroxide and dilute perchloric acid and finally determined immediately after the spectroscopic measurements. Optical rotations of amino acids were measured on a Yanagimoto OR-10 polarimeter at 589 nm in a 5-cm quartz cell at 20 ±0.1 °C. Infrared spectra of the isolated mixed ligand copper(II) complexes were obtained in the range 4000—650 cm^{-1} with a Hitachi 215 grating infrared spectrophotometer with the KBr disk method.

Optical Resolution of Racemic Aspartic Acid and Glutamic Acid. For every pair of DL-A and L-B, 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 sodium 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-argH)]·$2H_2O$ 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 1×100 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 3M 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 recrystallized once 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-A : DL-B molar ratio of 1 : 1 : 1.5 to avoid precipitation of less soluble binary complexes $Cu(B)_2$. The amino acids B incorporated into Cu(L-A)(B) were separated from L-A through a 1×100 cm column of Amberlite IR-45 in the Cl^- form by eluting with water and obtained as hydrochlorides.

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-B) and Cu(L-A)(B) systems with five different enantiomer contents of A and B, 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-B) and Cu(L-A)(B) 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-B) systems changed only slightly at different L- or D-Asp contents, the calibration curves were made by plotting the enantiomer contents against the maximum wavelengths that shifted with the contents.

Results and Discussion

Stereoselective Incorporation of Amino Acids into Ternary Complexes. Yields and optical purities of the acidic and the basic amino acids obtained *via* the ternary complex formation are summarized in Tables 1 and 2. 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-A or an L-B. Another line of evidence supporting the incorporation of the D-enantiomers is given by the optical purities of A and B estimated directly from the CD spectra of the complexes in the d-d region according to the calibration curves, such as are shown in Figs. 1 and 2. The optical purities determined by the two methods are in reasonable agreement with each other, and the differences between the correspoding 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-B)], showed the patterns that were more closely related to [Cu(D-A)(L-B)] than to [Cu(L-A)(L-B)], 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[10,11] have revealed that some bis(amino acidato)-

Table 1. Yields and optical purities of the D-enantiomers of acidic amino acids isolated *via* formation of the ternary complexes, Cu(A)(L-B)

Ligand A	Ligand B	Cu(A)(L-B) isolated: Yield (%)[a]	Cu(A)(L-B) isolated: Optical purity (%)[b]	A isolated: Yield (%)[c]	A isolated: 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 A estimated from the calibration curves shown in Fig. 1. c) Yield of isolated A based on the half amount of DL-A used. d) Estimated from the specific rotation, $[\alpha]_{589}^{20}$ (in 3 M HCl; $c=1$).

Table 2. Yields and optical purities of the D-enantiomers of basic amino acids isolated *via* formation of the ternary complexes, Cu(L-A)(B)

Ligand B	Ligand A	Cu(L-A)(B) isolated: Yield (%)[a]	Cu(L-A)(B) isolated: Optical purity (%)[b]	B isolated: Yield (%)[c]	B isolated: 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 B estimated from the calibration curves shown in Fig. 2. c) Yield of isolated B based on two-thirds of the amount of DL-B used. d) Estimated from the specific rotation, $[\alpha]_{589}^{20}$ (in 3 M HCl; $c=1$).

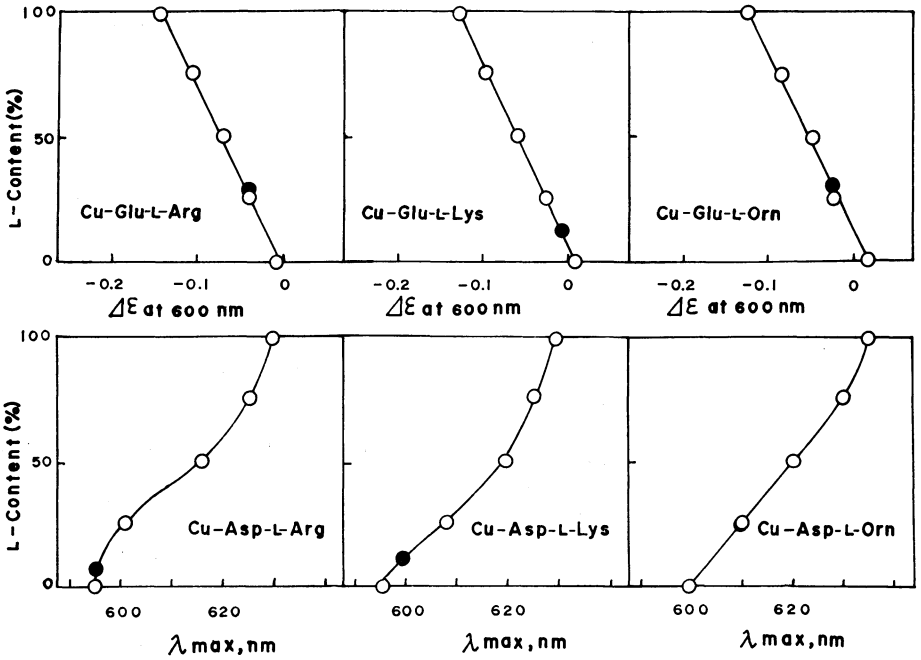

Fig. 1. Determination of the optical purities of acidic amino acids incorporated into Cu(A)(L-B) (●) from the calibration curves based on the CD spectra of the standard samples (○).

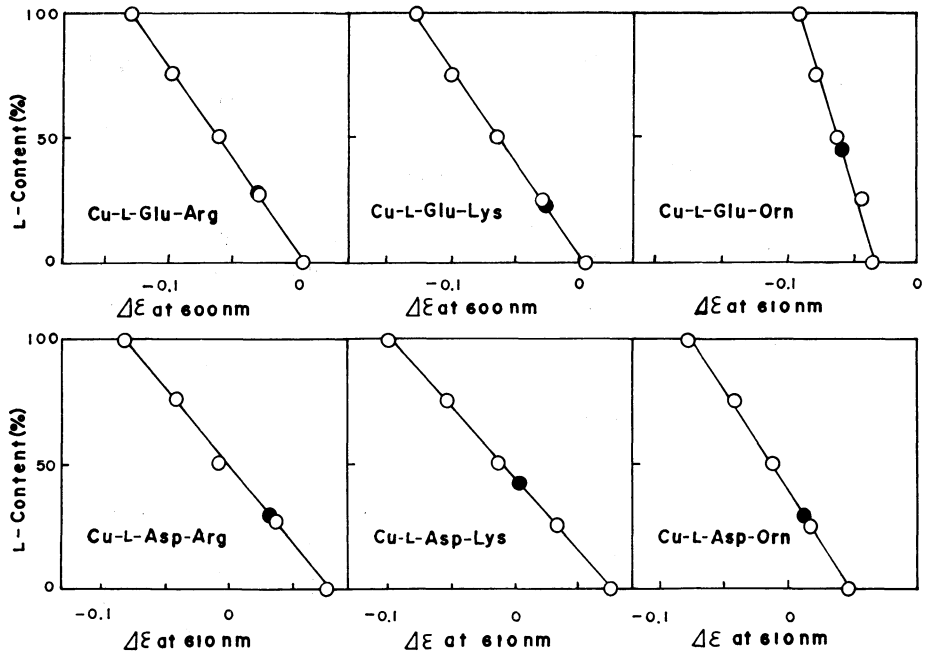

Fig. 2. Determination of the optical purities of basic amino acids incorporated into Cu(L-A)(B) (●) from the calibration curves based on the CD spectra of the standard samples (○).

copper(II) complexes assume a *cis* structure while others assume a *trans* one, every possible combination of A and B invariably gives as the main product a *meso* complex probably with a *cis* structure.[6a]

Factors Affecting the Optical Resolution. Probably owing to the electrostatic ligand-ligand interactions, the protonated ternary copper(II) complexes containing L-histidine and a basic amino acid, such as L-arginine and L-lysine, have been reported to have slightly higher stability constants than those containing D-histidine in place of L-histidine.[2b,2c] Although equilibrium constants are a reliable source of information about the species distribution and hence the stereoselectivity in solution, it seems difficult to detect the selectivity in solution in the present cases, because the electrostatic ligand-ligand interactions are seriously affected by the ionic strength of the solution, whose influence has been found to be reflected in the CD magnitudes of the

ternary systems.[5,6] A preliminary potentiometric study showed that the enantiomeric pairs of the ternary systems give almost identical titration curves at the ionic strength of 0.1(KNO_3). 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-A, and L-B, which indicates that there exists no remarkable preference of *cis*-[Cu(D-A)(L-B)] over *trans*-[Cu(L-A)(L-B)] 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 had incorporated D-Orn with 17% enantiomeric excess. These findings suggest that the solubility factor rather than the stability factor plays an 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, and the resulting *cis-trans* isomerism makes optical resolution of racemic ligands feasible under favorable conditions.

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