Journal of Chromatography, 239 (1982) 451–462
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 14,550

RESOLUTION OF OPTICAL ISOMERS OF Dns-AMINO ACIDS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH L-HISTIDINE AND ITS DERIVATIVES IN THE MOBILE PHASE

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SUMMARY

This paper describes our continuing work in resolving the optical isomers of Dns-amino acids by high-performance liquid chromatography with mixed chelate complexation. Addition of copper(II) complexes of L-histidine to the mobile phase resulted in resolution of many D- and L-Dns-amino acids, including those with aliphatic, polar and aromatic substituents. With polar substituents, highly selective incorporation of the L-enantiomer into the ternary complex with increased retention on the column was observed. The reverse occurred with amino acids with aliphatic side chains; the D-isomers were incorporated preferentially. With aromatic substituted amino acids, the resolution was pH dependent. Substitution of copper(II) complexes of L-histidine methyl ester in the mobile phase dramatically reduced the stereoselectivity, although the isomers were still resolved. Copper(II) complexes of N-acetyl-L-histidine used in the mobile phase gave no stereoselectivity.

Excellent separations of isomers were achieved with several of these systems. Many pairs of amino acids could be separated in the same chromatographic analysis.

INTRODUCTION

A number of approaches for resolving optical isomers of amino acids have been proposed[1–3]. In most of these, resolution is generally based on the different behavior of the isomers in ligand exchange, crown ether complexation, charge transfer complexation, hydrogen bonding interaction or metal complex formation.

In this technique, a non-polar, reversed-phase column is usually used with a mobile phase containing chiral metal complexes. For example, Karger and co-workers[4,5] used L-2-alkyl-4-octyldiethylenetriamine complexes of zinc and other metals in the mobile phase to separate Dns derivatives of the amino acids. Hare and Gil-Av[6], who previously reported using Cu(II)–proline eluents to separate free D- and L-amino acids, more recently studied Cu(II)–di-N-propyl-alanine complexes[7]. Grushka and co-workers[8,9], using complexes of aspartame and derivatives of aspartic acid, resolved a number of D- and L-amino acids.

We previously reported separations accomplished with Cu(II)–L-proline and

Cu(II)–L-arginine eluents[1,10,11]. This paper describes our experience with copper(II) complexes of L-histidine and L-histidine derivatives.

Histidine forms some of the most important metal-binding sites in such proteins as carboxypeptidase A[12], thermolysin[13], carbonic anhydrase B[14]. It has also been implicated in many other enzymes and metalloproteins and in copper(II) transport in blood[15,16], although the manner in which histidine and its derivatives participate in complex formation is still uncertain.

Metal complexes of histidine and its esters form mixed complexes with other amino acids with marked stereoselectivity. The first evidence of this came from a nuclear magnetic resonance (NMR) study of Co(II)–histidine complexes[17]. Subsequently, Brookes and Pettit[18] demonstrated stereoselectivity in Cu(II) complexes of L-histidine and amino acids with a polar side group.

EXPERIMENTAL

Instrumentation

The chromatograph was a Perkin-Elmer Series 2 LC equipped with a Rheodyne 7105 injection valve, a Model LC 650-10 fluorescence spectrophotometer and a Model 56 chart recorder (Perkin Elmer, Norwalk, CT, U.S.A.). Fluorescence of the Dns derivatives at 480 nm was monitored with excitation at 340 nm. The analytical columns were 15 × 0.42 cm packed with Spherisorb® C_{18} (Phase Separations, Hauppage, NY, U.S.A.). The columns were packed by the downward slurry technique.

Reagents

Acetonitrile distilled-in-glass was bought from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). D- and L-Dns-amino acids from Sigma (St. Louis, MO, U.S.A.) and Pierce (Rockford, IL, U.S.A.). Other Dns-amino acids were prepared as previously described[1]. The mobile phases in general contained 10–20% of acetonitrile in a buffer that was $5.0 \cdot 10^{-3}$ *M* of an optically active amino acid, $2.5 \cdot 10^{-3}$ *M* copper sulfate and 2.0 g of ammonium acetate.

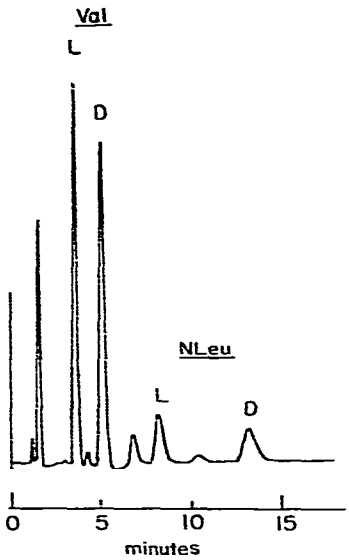
RESULTS

A mobile phase containing L-histidine and Cu(II) in a 2:1 molar ratio resolved Dns-amino acids with aliphatic, aromatic and polar substituents (Table I). The selectivity was excellent; low-molecular-weight amino acids were completely separated (Figs. 1–3).

As in other reversed-phase analyses, the separation depended on the acetonitrile concentration in the mobile phase (Table I). With decreasing acetonitrile concentration, solutes were retained longer, and the resolution and selectivity improved (Table II).

Esterification of the carboxylate function also had a marked effect (Table III, Figs. 4–6).

Because of competition for binding sites by hydrogen ions, the ability of the amino acid to bind Cu(II) decreased at lower pH, with an effect on both selectivity and capacity factor (Tables IV and V). With L-histidine at pH 5, there was complete



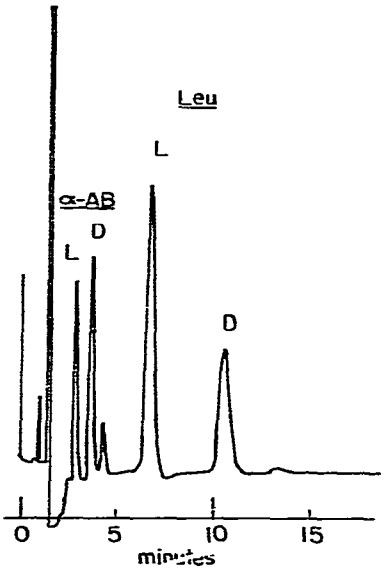


Fig. 1. Separation of D,L-Dns-valine and norleucine with L-histidine–Cu(II) eluent. Mobile phase: 17.5% acetonitrile in an aqueous solution containing 5 m*M* L-histidine, 2.5 m*M* $CuSO_4 \cdot 5H_2O$ and 2.0 g ammonium acetate, pH 7.0. Flow-rate: 2.0 ml/min.

Fig. 2. Separation of D,L-Dns-α-amino butyric acid and leucine. Conditions as in Fig. 1.

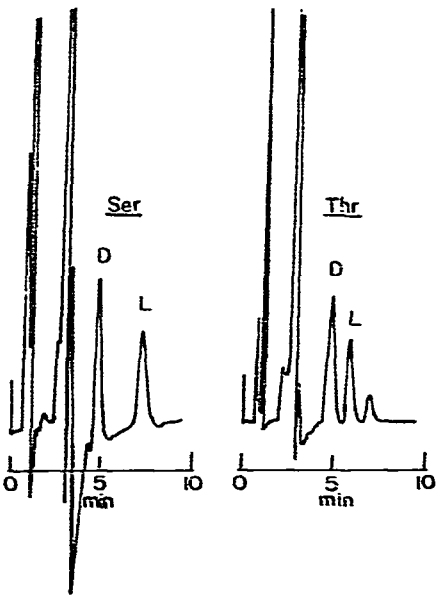


Fig. 3. Separation of D,L-Dns-serine and D,L-Dns-threonine with L-histidine–Cu(II) eluent. Mobile phase: 10% acetonitrile in an aqueous solution containing 5 m*M* L-histidine, 2.5 m*M* $CuSO_4 \cdot 5H_2O$ and 2.0 g ammonium acetate, pH 7.0. Flow-rate: 2.0 ml/min.

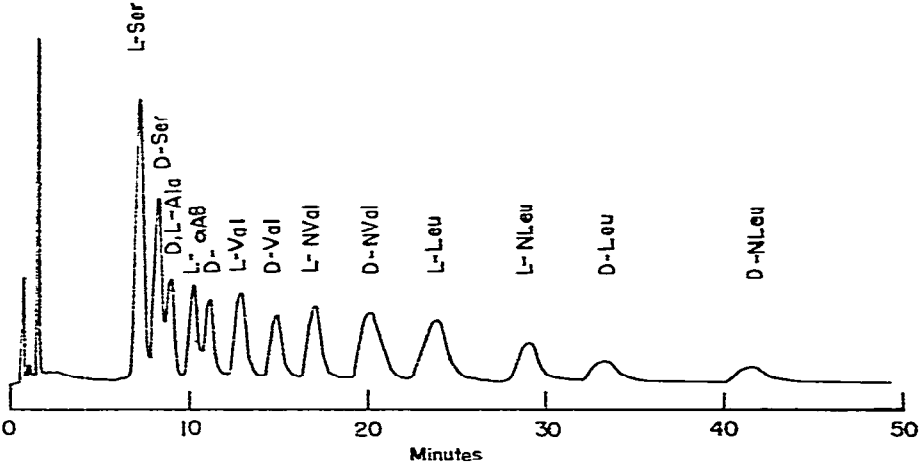


Fig. 4. Separation of D,L-Dns-amino acids with Cu(II)–L-histidine methyl ester eluent. Mobile phase: 20% acetonitrile in an aqueous solution containing 5 m*M* L-histidine methyl ester, 2.5 m*M* $CuSO_4 \cdot 5H_2O$ and 2.0 g ammonium acetate, pH 7.0. Flow-rate: 2.0 ml/min.

loss of resolution for most of the amino acids while isomers of tryptophan and phenylalanine were separated more. With the L-histidine methyl ester, there was decreased selectivity with decrease in pH, although some separation of optical isomers persisted.

TABLE I

CAPACITY RATIO (k') AND SELECTIVITY (α) OF D- AND L-Dns AMINO ACIDS

Mobile phase: aqueous solution containing 5 m*M* L-histidine, 2.5 m*M* $CuSO_4 \cdot 5H_2O$ and 2.0 g ammonium acetate,l of deionized water with the following percentage of acetonitrile: (1–3) 10%; (5–12) 15%. pH 7.0. Flow-rate: 2.0 ml/min.

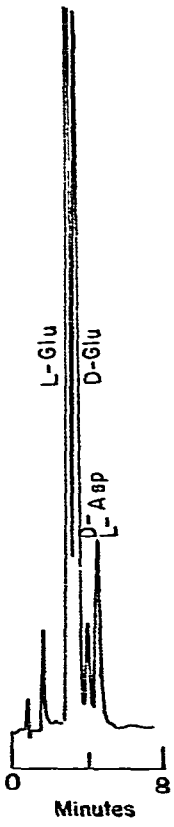
No.	*Amino acid*	k'_L	k'_D	α
1	Thr	4.4	3.4	0.77
2	Ser	5.6	3.4	0.61
3	Ala	6.6	8.2	1.2
4	α-AB	2.2	3.8	1.7
5	Val	3.2	6.4	2.0
6	Met	4.4	7.8	1.8
7	NVal	3.8	9.0	2.4
8	ILeu	7.2	15.4	2.1
9	Leu	7.2	16.0	2.2
10	NLeu	9.4	22.2	2.4
11	Phe	9.0	9.0	1.0
12	Trp	7.4	7.4	1.0

TABLE II

CAPACITY RATIO (k') AND SELECTIVITY (α) AS A FUNCTION OF ACETONITRILE CONCENTRATION

Conditions as in Table I.

Amino acid	*20% Acetonitrile*			*17.5% Acetonitrile*			*15% Acetonitrile*		
	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α
α-AB	0.6	1.2	2.0	1.8	2.6	1.4	2.2	3.8	1.7
Val	1.2	2.0	1.7	2.6	4.0	1.5	3.2	6.4	2.0
Met	1.2	2.0	1.7	3.8	5.4	1.4	4.4	7.8	1.8
NVal	1.4	2.6	1.9	3.2	5.8	1.8	3.8	9.0	2.4
ILeu	2.6	4.6	1.8	5.6	9.0	1.6	7.2	15.4	2.1
Leu	2.6	4.8	1.8	5.6	9.6	1.7	7.2	16.0	2.2
NLeu	3.0	5.8	1.9	7.0	11.8	1.7	9.4	22.2	2.4
Phe	2.6	2.6	1.0	5.4	5.4	1.0	9.0	9.0	1.0
Trp	2.2	2.2	1.0	4.8	4.8	1.0	7.4	7.4	1.0



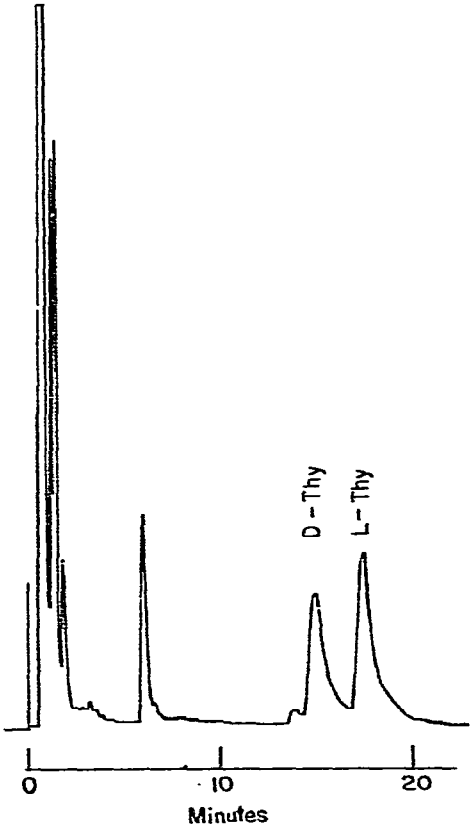


Fig. 5. Separation of D,L-Dns-glutamic acid and aspartic acids. Conditions as in Fig. 4.

Fig. 6. Separation of D,L-Dns-thyroxine with Cu(II)–L-histidine methyl ester eluent. Mobile phase: 40% acetonitrile in an aqueous solution containing 5 m*M* L-histidine methyl ester, 2.5 m*M* $CuSO_4 \cdot 5H_2O$ and 2.0 g ammonium acetate, pH 7.0. Flow-rate: 2.0 ml/min.

TABLE III

CAPACITY RATIO (k') AND SELECTIVITY (α) OF D- AND L-Dns-AMINO ACIDS

Mobile phase: 20.0% acetonitrile in an aqueous solution containing 5 m*M* L-histidine methyl ester, 2.5 m*M* $CuSO_4 \cdot 5H_2O$, and 2.0 g ammonium acetate/l of deionized water. pH 7.0. Flow-rate: 2.0 ml/min.

Amino acid	k'_L	k'_D	α
Glu	3.3	4.1	1.3
Asp	5.0	4.4	0.88
Asn	7.9	8.7	1.1
Ser	9.3	10.7	1.2
Thr	9.3	9.3	1.0
Ala	11.9	11.9	1.0
α-AB	13.6	15.0	1.1
Val	17.3	20.1	1.2
Met	27.2	30.7	1.1
NVal	23.3	27.6	1.2
ILeu	40.4	44.7	1.1
Leu	33.0	43.9	1.3
NLeu	40.4	58.4	1.5
Phe	77.9	60.4	0.78
Trp	121.8	93.3	0.77
Thy*	21.5	20.4	0.95

* 40% Acetonitrile.

TABLE IV

CAPACITY RATIO (k') AND SELECTIVITY (α) OF D- AND L-Dns-AMINO ACIDS AS A FUNCTION OF pH

Mobile phase: 15.0% acetonitrile in an aqueous solution containing 5 m*M* L-histidine, 2.5 m*M* $CuSO_4 \cdot 5H_2O$, and 2.0 g ammonium acetate/l of deionized water. Flow-rate: 2.0 ml/min.

Amino acid	*pH 7*			*pH 5*		
	k'_L	k'_D	α	k'_L	k'_D	α
α-AB	2.2	3.8	1.7	8.4	8.4	1.0
Val	3.2	6.4	2.0	13.8	13.8	1.0
Met	4.4	7.8	1.8	14.4	14.4	1.0
NVal	3.8	9.0	2.4	16.4	16.4	1.0
ILeu	7.2	15.4	2.1	30.4	30.4	1.0
Leu	7.2	16.0	2.2	31.4	31.4	1.0
NLeu	9.4	22.2	2.4	37.0	37.0	1.0
Phe	9.0	9.0	1.0	34.0	30.0	0.88
Trp	7.4	7.4	1.0	34.0	27.0	0.79

TABLE V

CAPACITY RATIO (k') AND SELECTIVITY (α) OF D- AND L-Dns-AMINO ACIDS

Mobile phase: 20.0% acetonitrile in an aqueous solution containing 5 m*M* L-histidine methyl ester, 2.5 m*M* $CuSO_4 \cdot 5H_2O$, and 2.0 g ammonium acetate/l of deionized water. pH as indicated in table. Flow-rate: 2.0 ml/min.

Amino acid	*pH 7*			*pH 5*		
	k'_L	k'_D	α	k'_L	k'_D	α
Glu	3.3	4.1	1.3	5.0	5.6	1.1
Asp	5.0	4.4	0.88	6.7	6.1	0.91
Asn	7.9	8.7	1.1	9.6	11.3	1.2
Ser	9.3	10.7	1.2	7.8	9.0	1.2
Thr	9.3	9.3	1.0	9.0	9.0	1.0
Ala	11.9	11.9	1.0	10.7	10.7	1.0
α-AB	13.6	15.0	1.1	10.7	11.3	1.1
Val	17.3	20.1	1.2	16.1	17.0	1.1
Met	27.2	30.7	1.1	20.4	22.4	1.1
NVal	23.3	27.6	1.2	19.3	21.3	1.1
Leu	33.0	43.9	1.3	31.3	34.1	1.1
NLeu	40.4	58.4	1.5	39.0	43.6	1.1
Phe	77.9	60.4	0.78	66.1	53.9	0.81
Trp	121.8	93.3	0.77	100.7	81.0	0.81

TABLE VI

COMPARISON OF SELECTIVITY (α) AND CAPACITY RATIO (k') BETWEEN FOUR ELUENT SYSTEMS CONTAINING Cu(II) COMPLEXES OF THE L-AMINO ACIDS SHOWN IN THE HEADINGS

Acetonitrile concentration was 15% for proline and histidine system and 20% for histidine methyl ester and arginine system. Other conditions as in Table I.

	Proline			*Histidine*			*Histidine methyl ester*			*Arginine (ref. 1)*		
	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_D	k'_L	α
Ser	3.7	3.2	0.87	—	—	—	9.3	10.7	1.2	3.0	3.0	1.0
Thr	3.7	4.6	1.2	—	—	—	9.3	9.3	1.0	3.0	3.0	1.0
Ala	5.7	6.6	1.2	1.8	2.4	1.3	11.9	11.9	1.0	4.2	4.4	1.1
α-AB	7.3	9.2	1.2	2.2	3.8	1.7	13.6	15.0	1.1	5.7	6.2	1.1
Val	11.4	15.0	1.3	3.2	6.4	2.0	17.3	20.1	1.2	8.3	9.5	1.1
Met	11.4	14.8	1.3	4.4	7.8	1.8	27.2	30.7	1.1	10.3	11.7	1.1
NVal	14.6	19.0	1.3	3.8	9.0	2.4	23.3	27.6	1.2	11.0	13.0	1.2
iLeu	—	—	—	7.2	15.4	2.1	40.4	44.7	1.1	17.2	20.8	1.2
Leu	23.9	32.6	1.4	7.2	16.0	2.2	33.0	43.9	1.3	18.5	20.8	1.1
NLeu	32.6	45.7	1.4	9.4	22.2	2.4	40.4	58.4	1.5	22.3	28.2	1.3
Phe	32.6	52.8	1.6	9.0	9.0	1.0	77.9	60.4	0.78	20.3	23.0	1.1
Trp	41.2	71.2	1.7	7.4	7.4	1.0	121.8	93.3	0.77	24.8	31.2	1.3

DISCUSSION

When a divalent metal ion, M(II), is in equilibrium with 2 different amino acids, Ax and Ay, both of which can form complexes with the metal ion, the following equilibria are present:

$$M + 2Ax \rightleftharpoons M(Ax)_2$$
$$M + 2Ay \rightleftharpoons M(Ay)_2$$
$$M + Ax + Ay \rightleftharpoons M(Ax)(Ay)$$
$$M(Ax)_2 + M(Ay)_2 \rightleftharpoons 2M(Ax)(Ay)$$

The disproportionation constant K can be written as:

$$K = [M(Ax)(Ay)]^2/[M(Ax)_2][M(Ay)_2]$$

Statistically there are two ways the mixed complex M(Ax) (Ay) can be formed but only one way the binary complexes $M(Ax)_2$ and $M(Ay)_2$ can be. The expected disproportionation constant is 4.

When the stability constants for the mixed complex M(Ax)(Ay) and for the binary complexes $M(Ax)_2$ and $M(Ay)_2$ are determined, following Siegel[19], it is possible to define the stability of the ternary complex as:

$$\log K = 2 \log_{M(Ax)(Ay)} - \log_{M(Ax)_2} - \log_{M(Ay)_2}$$

Thus the tendency towards ternary complex formation can be characterized by the sign and the value of log K. The expected value of a favorable disproportionation reaction is log 4, or 0.6. A value of log K of greater than 0.6 suggests stabilization of the ternary complex.

The formation constants of some ternary complexes of Cu(II)–L-histidine and bidentate amino acids, measured by potentiometric methods[18] are as follows:

Cu(II)–L-His–L-amino acid	L-Phe	L-Try	L-Val	L-Leu	L-Thr	L-Ser
log K	2.10	2.37	2.20	2.13	2.24	1.90

The log K values greater than 0.6 suggest that histidine does indeed form ternary complexes. It has also been shown that in the Cu(II)–L-histidine–L-bidentate amino acid system over 70% of the metal complexes are ternary and with the remaining 30% distributed among the others[20].

Based on these findings, we used the binary complex of Cu(II)–L-histidine and Cu(II)–L-histidine methyl ester as the mobile phase in reversed-phase chromatography. We postulated that on the introduction of an amino acid an equilibrium of the parent complex and the ternary complex would be established; the charged species and the metal ions would remain in the aqueous phase, while the more hydrophobic and neutral binary and ternary complexes would partition into the non-polar stationary phase. Since a chiral metal complex was to be used, different disproportionation and stereoselectivity was expected with enantiomeric solutes. If one of the two

optical isomers of the solute amino acid were to displace histidine from the binary complex more strongly, separation in the high-performance liquid chromatographic (HPLC) system would have become possible.

Brookes and Pettit[18], using potentiometric measurements, demonstrated a significant degree of stereoselectivity in the formation of the ternary complex of Cu(II), L-histidine and amino acids with polar groups in the side chain. Using Cu(II)–L-histidine complexes in the mobile phase, we achieved optical resolution of Dns-amino acids with aliphatic as well as polar side-chains.

The order of elution generally was consistent with other reversed-phase separations: the higher the carbon content, the bulkier the alkyl substituent on the α-carbon (Fig. 1), the longer the retention. With isomers with equal numbers of carbons such as norleucine and leucine, the straight chain isomer was retained more, presumably because of stronger interaction with the stationary phase. The selectivity between the D- and L-pairs was also affected by the bulkiness of the alkyl substituents.

The stereoselectivity followed a defined pattern (Table I). The L-isomers of those amino acids with aliphatic side-chains eluted before the D-isomers. The D-isomers of amino acids with chelatable side chains were retarded more, yielding a greater degree of separation, while no resolution was observed with amino acids with charge transfer groups. Yamauchi *et al.*[20] showed that L-histidine and amino acids with polar, chelatable side-chains formed ternary complexes such as is shown in Fig. 7. The polar side-chain of the coordinating amino acid, represented by "X", is in the vicinity of the oxygen of the apically coordinated carboxyl group. Stabilization of the ternary complex by hydrogen bonding is probable. Hydrogen bonding is a weak but significant driving force for the stereospecific formation of the more stable L-His–L-amino acid complex with the polar side group. The retention behavior and selectivity of the polar amino acids, serine and threonine, are consistent with the above model, involving intramolecular hydrogen bonding of the terdentate histidine and bidentate amino acids coordinated around copper(II) in a *cis* configuration. With amino acids with aliphatic side-chains, hydrogen bonding interaction is not present. Instead the carboxyl group at the apical position has more steric interaction with the hydrocarbon part of the ternary complex and interferes with the approach of the carboxylate oxygen to the apical coordination site. This causes the meso complex, (L-His)–Cu(II)–(D-amino acid), to be more stable and retained longer.

Separation of the D- and L-pairs also depends on the alkyl substituent on the α-carbon. With the exception of phenylalanine and tryptophan, the bulkier the alkyl group, the higher the selectivity factor (α). Although the separation of the stereoisomers of these two amino acids was greatest in all of the other systems we

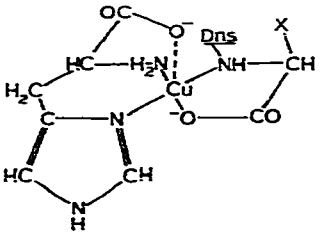


Fig. 7. Structure of L-His–Cu(II)–L-amino acid ternary complex.

studied, including the proline, arginine and histidine methyl ester systems, there was no stereoselectivity with the free histidine system at pH 7. With the Dns-derivative of phenylalanine and tryptophan, pi–pi interaction with the imidazole ring of histidine is possible. Charge transfer interaction can occur with the imidazole of histidine and the naphthyl and phenyl group of Dns-phenylalanine or the naphthyl and indole groups of Dns-tryptophan. Construction of a three-dimensional model shows that a hydrocarbon cluster is formed above the coordination plane with the amino acids bonded to the copper in the same way as is glycine; between carboxyl and α-amino nitrogen. The formation of such a complex is evidently possible with both D- and L-isomers, with no difference in the stability of the complex.

Urry and Eyring[21] have shown that histidine methyl ester would coordinate around a metal ion in a terdentate manner with the acyl oxygen at the apex. Using Cu(II)–L-histidine methyl ester as the eluent, we observed stereoselective formation of metal complexes and resolution of many pairs of Dns-amino acids (Table III, Figs. 4 and 5). The D-isomers are generally retained longer; even those such as serine with polar side-chains. The complexation mechanism in this case no longer follows the pattern of free histidine. The apical carbonyl makes no contribution to complex stability through hydrogen bonding with the weak hydrogen donors of the polar amino acids. In this case, steric interaction is more important in determining the stability of the complex. However, with the amino acids studied, reversed stereoselectivity was observed for aspartic acid, the aromatic acids, phenylalanine, thyroxine and the heterocyclic amino acid, tryptophan.

This reversed selectivity of aspartic, compared to glutamic, suggests that these two amino acids bond differently with the L-histidine methyl ester and supports the idea that glutamic acid bonds the way glycine does, while aspartic acid is terdentate[18] with hydrogen bonding of the carboxylic group of aspartic and the carbonyl group of histidine methyl ester resulting in the observed reversal in selectivity.

The Dns-phenylalanine and tryptophan optical isomers, not resolved by the free histidine system (Table I), were separated by the Cu(II)–histidine methyl ester mobile phase (Table III). The stereoselectivity, however, was not as expected from that reported for the free histidine in solution[18]. Here the ternary complex formed by the ligands of the same chirality was more stable and more strongly retained. With methylation of the carboxylate group of histidine, the histidine derivative can no longer bond as does glycine as in the free histidine system, thus preventing the formation of clusters by the naphthyl, the aromatic and the imidazole groups. Instead, the histidine methyl ester with its amine and imidazole group bonded in the *trans* manner, with the carbonyl weakly coordinated on the apical site, and the aromatic ring on the opposite side of the carbonyl. Therefore, the meso complex having both carbonyl and the ring system competing for the same coordination site is less stable. Phenylalanine and tryptophan with the aromatic ring in the side-chain have been demonstrated to undergo pi–pi interaction with the Cu(II) ion[22]. The amino acid in this case can be regarded as a terdentate ligand.

The histidine molecule offers three potential coordination sites in aqueous solution: the carboxyl group ($pK_a = 1.9$), the imidazole nitrogen ($pK_a = 6.1$) and the amino nitrogen ($pK_a = 9.1$)[23]; while the histidine methyl ester has two coordination sites, the imidazole nitrogen ($pK_a = 5.4$) and the amino nitrogen ($pK_a = 7.3$)[24] becoming available for complexation as pH increases. A change in the pH of the

mobile phase would result in a change in the equilibrium distribution of the various metal species in the solution. With the lowering of pH, the hydrogen ion would compete with the Cu(II) ion for the chelating sites. The resolution would diminish (Tables IV and V). At pH 7, more than 70% of the metal complex is present. At lower pH, this percentage decreases. It is important to note that both the amino and the imidazole nitrogen are very effective bidentate ligands at pH 5. Significant resolution of the Dns-amino acids is observed (Table V). This can be due to the fact that histidine methyl ester is more hydrophobic, as is evident from the acetonitrile concentration needed in the mobile phase to elute of the amino acids (Table III). Histidine methyl ester also has smaller dissociation constants. At pH 5 appreciable portions of the metal complexes of the L-histidine methyl ester are distributed in the stationary phase.

The free histidine system at pH 5 gave no stereoselectivity for most amino acids but resolved tryptophan and phenylalanine isomers for the first time (Table IV). At this pH, the histidine complex may have bonded in the same manner as that of the histidine methyl ester, as suggested by the similar selectivity and orders of elution. The carboxylate molecule may be involved in hydrogen bonding with H^+ or water molecules, thus rendering it unavailable for glycine-like coordination.

With copper(II) complexes of N-acetyl-L-histidine under the same mobile phase conditions, no resolution of the Dns-amino acid optical isomers was observed. As noted for the histidine methyl ester system, the amino group chelates the metal effectively. Blocking of the functional group by acylation resulted in a weaker complex and loss of stereoselectivity.

Table VI compares the selectivity and capacity factors of several systems. Even though the acetonitrile concentrations used with the four systems were different, the stereoselectivity was affected only slightly (Table II and ref. 1). Histidine differs from other amino acids in that it possesses a bulky substituent on the α-carbon. It also functions as a terdentate ligand which gives the mixed complex a rigid conformation, and thus high stereoselectivity. Histidine methyl ester gives a less rigid complex, with reduced selectivity. Proline has an intermediate selectivity because it has a ring system standing above the coordination plane, unlike the imidazole ring of histidine methyl ester which lies flat on the plane. Arginine which does not have a rigid ring structure gives selectivity similar to histidine methyl ester. Therefore, the three-dimensional conformation of the ternary complex is important in deciding stereoselectivity. Stronger spatial interaction results in better optical resolution.

Histidine methyl ester and arginine systems offer adequate stereoselectivity and excellent separation of the different Dns-amino acids while histidine offers excellent stereoselectivity for the enantiomers but poor separations of the amino acids. It is possible to optimize the histidine methyl ester or the arginine system for a one-column system in the separation of most of the amino acids into their respective isomers by some form of mobile phase gradient.

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