

Enantiomeric Separation of Free Amino Acids Using N-alkyl-L-proline Copper(II) Complex as Chiral Mobile Phase Additive in Reversed Phase Liquid Chromatography

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Enantiomeric separation of free amino acids has been achieved by a reversed phase liquid chromatography with addition of a Cu(II) complex of N-alkyl-L-proline (alkyl: propyl, pentyl or octyl) to the mobile phase. The amino acids eluted were detected by a postcolumn OPA system. N-alkyl-L-proline was prepared and used as a chiral ligand of Cu(II) chelate for the enantiomeric separation. The concentration of the Cu(II) chelate, the organic modifier and pH affect the enantiomeric separation of free amino acids. The retention behaviour, varied with change in pH and the concentration of the Cu(II) chelate, was different compared with those of the derivatized amino acids. The elution orders between D- and L- forms were consistent except histidine showing that L-forms elute earlier than D-forms. The retention mechanism for the enantiomeric separation can be illustrated by the stereospecificity of the ligand exchange reaction and the hydrophobic interaction between the substituent of amino acids and reversed phase, C₁₈.

Introduction

High performance liquid chromatography (HPLC) is a technique that has proven useful in both the analysis and the purification of biological molecules. The ability of modern HPLC instrumentation to rapidly separate complex mixtures offers the investigator a technology ideally suited to the analysis of amino acids.¹⁻⁵ Stationary phase chemistries and detection systems can be optimized for specific analytical requirements.

The first chiral chelate packing materials became commercially available in 1983 with the production of silica gels modified with L-proline or L-valine by Serva, West Germany. Before this, since experience was needed to produce the chiral sorbents giving acceptable performance, ligand exchange chromatography (LEC) of enantiomers was the privilege of a limited number of research groups having a certain level of experience in macromolecular chemistry or silica gel modification. To overcome this handicap, the addition of chiral metal complexes to the eluent was suggested in 1979 as a novel approach to LEC of enantiomers.^{6,7} The method of chiral eluent relates to an early suggestion⁸ by Cram and coworkers to resolve racemic amino acid esters on celite or alumina by adding a chiral crown ether to the eluent. The method permits one to resolve racemic compounds using conventional achiral column packing. However, some possible detection problems caused by the presence of a chiral background in the effluent should be taken into account.

It has been found to be useful to react the amino acid functionality common to all amino acids with a derivatizing reagent to yield a product that can be detected both specifically and with high sensitivity. The chemical structure of the derivatized product will determine the detection system. Most commonly, the derivatives chosen will either fluoresce or absorb light in the ultraviolet or visible region. Precolumn derivatization of amino acids such as dansylation or dansylation that produce very bulky molecules hinders the complexation and enhance hydrophobic interaction with the stationary phase. In the case of free amino acids, modification

Table 1. Reports for Enantiomeric Separation of Free Amino Acids Using Chiral Mobile Phase Additive

Chiral resolving ligand	Metal ion	Racemate resolved, max. selectivity	Elution order of enantiomers	Ref.
N,N-dimethyl-L-leucine	Cu ²⁺	10 amino acids	D, L	14
N,N-dipropyl-L-alanine	Cu ²⁺	19 amino acids $\alpha=2.7$ (Leu)	D, L	10, 11, 12
N,N-dimethyl-D-phenylglycine	Cu ²⁺	5 amino acids	L, D	13
L-proline	Cu ²⁺	19 amino acids $\alpha=6.5$ (Val)	D, L except His, Asp, Thr	9
N-methyl-L-proline	Cu ²⁺	Ala, Val, <i>t</i> -Leu, Tyr, Abu	D, L	13
N-benzyl-L-proline	Cu ²⁺	Val, Pro, Hyp $\alpha=3.7$ (Pro)	L, D except aHyp	15
L-proline	Cu ²⁺	23 amino acids $\alpha=1.3$ (Tyr)	D, L	1

of enantioselectivity and retention time was feasible because a ternary complexation and hydrophobic interaction between the side chain of amino acids and stationary phase affect the separation. Enantiomeric separation of amino acids has been extensively investigated as shown in Table 1. It has been found that the enantiomeric separation of free amino acids varies with the ligand of the complex and the elution orders between the enantiomeric pairs are also different. It is of great interest to see the difference of the enantiomeric separation by applying a new chiral complex system as a chiral mobile phase system.

A postcolumn derivatization producing fluorophores provides an alternative to the use of ninhydrin. Both *o*-phthalaldehyde (OPA) and fluorescamine have been used to react rapidly with primary amines to form intense fluorescent pro-

ducts.^{16,17} OPA has been used as the best detection fluorescent reagent that is relatively stable in the aqueous reaction during detection. The OPA detection of amino acids has been accomplished in a number of studies.¹⁸⁻²³

The objective of this research is to identify a new enantiomeric separation system having a new chiral resolving agent to resolve racemic amino acids. The N-substituted cyclic amino acids such as proline, hydroxyproline and allohydroxyproline in Cu(II) complexes were found to display the best enantioselectivity from a number of amino acid chelate studies.^{24,25} The best enantioselectivity comes from the their chelate ring's inflexibility due to the 5-membered pyrrolidone ring. Proline is also favored to use as chiral additive due to the good solubility (162 g/100 ml at 25°C) compared with the other amino acids (ca. several mg/100 ml at 25°C). In this paper, we prepared chiral copper(II) chelates involving N-alkyl-L-proline (alkyl: propyl, pentyl or octyl) as chiral additives to the mobile phase to see the resolution of the underivatized amino acid enantiomers. The mechanism of the enantiomeric separation of the free amino acids on the basis of a ligand exchange reaction as well as the hydrophobic interaction between the ternary complex and C₁₈ stationary phase has also been investigated and compared with that of the derivatized amino acids.

Experiment

Materials. Free amino acids such as alanine (Ala), valine (Val), leucine (Leu), isoleucine (Ile), phenylalanine (Phe), tryptophan (Trp), tyrosine (Tyr), proline (Pro), asparagine (Asn), glutamine (Gln), histidine (His), arginine (Arg), lysine (Lys), aspartic acid (Asp), glutamic acid (Glu), methionine (Met), serine (Ser) and threonine (Thr) were obtained from Sigma (St. Louis, MO, USA). O-phthalaldehyde (OPA) was also purchased from Sigma (St. Louis, MO, USA). Propyl bromide, pentyl bromide and octyl bromide were obtained from Aldrich (Milwaukee, WI, USA). Acetonitrile, dichloromethane, chloroform, methyl alcohol and ethyl alcohol were purchased from Merck (Darmstadt, F.R. Germany). Solvents for the mobile phase were HPLC grade. Triply distilled water was used for the preparation of mobile phase. Samples were prepared by dissolving the amino acids in the eluent to provide a concentration of approximately 10^{-3} M and sodium bicarbonate (0.1 M) was added. The prepared samples were directly introduced to the injector of the liquid chromatograph system for the separation.

Instrument. The Shimadzu Model LC-9A pump and Waters Model 510 pump were used to provide the mobile phase flow. Model U6K injector was used for the injection of the sample. Waters Model 420 fluorescence detector was employed to monitor column effluent. The chromatograms were recorded with Shimadzu Model C-R4A data station. The analytical column used was Spherisorb ODS-2 (Alltech, 150 mm × 4.6 mm, 3 μm). Fourier transform-infrared (FT-IR) spectrophotometer (Digilab, FTS-20B0, USA), Fourier transform-nuclear magnetic resonance (FT-NMR) spectrophotometer (Bruker, WP80-SY, Germany) and elemental analyzer (EA, Carlo Erba, 1106, Italy) were used to identify the alkyl prolines which were prepared. The wavelength of excitation and emission filters for the postcolumn detection of the OPA derivatized amino acids were 340 nm and 455 nm, respectively.

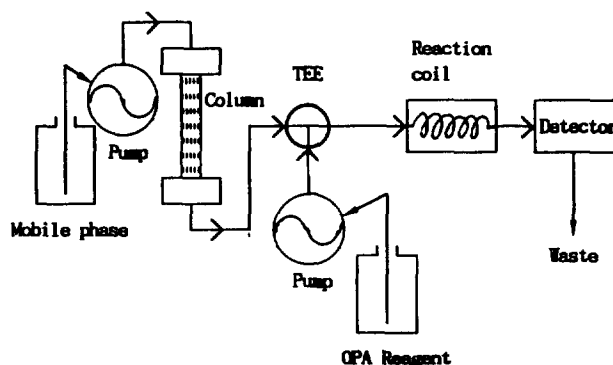


Figure 1. Diagram of postcolumn reaction system.

vely.

Preparation of Chiral Ligands. The ligand, N-alkyl-L-proline, was prepared by treating L-proline with alkyl bromide under a basic condition. 60 ml water containing 5.61 g (0.1 mole) potassium hydroxide and 11.51 g (0.1 mole) L-proline and 40 ml ethanol containing 0.12 M alkyl bromide were mixed and stirred for 5 hours with refluxing. After completion of the reaction, water and ethanol were evaporated by a rotary evaporator. The residue was dissolved in water. To remove the unreacted alkyl bromide, the aqueous solution was extracted with ethyl ether and the ethyl ether extracted was discarded. After removal of water in the extracted aqueous solution, the residue was dissolved in dichloromethane and then unreacted proline was precipitated. After filtration of proline, the product which was dissolved in dichloromethane was recrystallized by adding chloroform. The product was identified with FT-IR, FT-NMR and EA. The yield of N-propyl-L-proline, N-pentyl-L-proline and N-octyl-L-proline was 40%, 37% and 25%, respectively. N-alkyl-L-proline was used as the ligand for the copper chelate.

OPA Postcolumn Detection System. For the post-column detection, OPA reagent was prepared as follows.²⁶⁻³⁰ A 2.5 M aqueous solution (990 ml) of boric acid was adjusted to pH 10.4 with solid KOH and then 5 g EDTA added. In a separate container, 0.8 g OPA and 0.2 ml 2-mercaptoethanol was dissolved in 10 ml methanol. The both solutions were mixed in a bottle. The OPA reagent was stable at room temperature for at least a week in the dark.

For the postcolumn system as shown in Figure 1, the mobile phase was delivered at a constant flow rate of 1.0 ml/min. The OPA solution was delivered at a constant flow rate of 0.5 ml/min. The column effluent was mixed with the OPA solution in a T-piece. The mixture was allowed to flow and react successively through 150 cm long reaction coil (0.51 mm ID).

Mobile Phase Preparation. The mobile phase was prepared by dissolving cupric sulfate, N-alkyl-L-proline and buffer reagent in HPLC grade and then by adjusting the pH to the desired value with acetic acid or KOH. The required amount of the organic solvent (acetonitrile or methanol) can be added to the resulting aqueous solution.

Results and Discussion

A typical chromatogram for the separation of the free amino acid enantiomers is shown in Figure 2. As can be

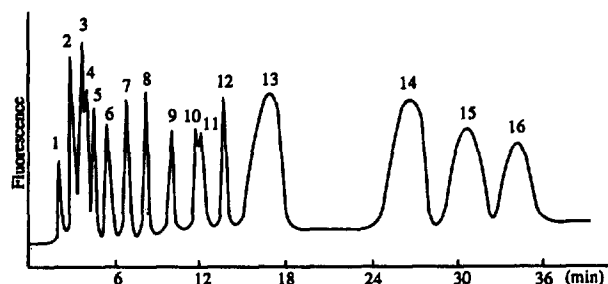


Figure 2. Chromatogram of free amino acids with OPA post-column detection. Mobile phase: 3.25×10^{-3} M Cu(N-pentyl-L-proline)₂, 1.0×10^{-2} M borate buffer and 1.0×10^{-3} M pyridine in aqueous solution at pH 5.5. Flow rate of mobile phase: 1.0 ml/min. Flow rate of OPA solution: 0.5 ml/min. Column: 15×0.46 cm, $3 \mu\text{m}$ (Spherisorb ODS-2). Elution sequence: 1. D-His 2. L-Ala 3. D-Ala 4. L-His 5. L-Glu 6. L-Val 7. D-Glu 8. L-Met 9. L-Ile 10. L-Leu 11. D-Val 12. L-Tyr 13. D-Met 14. D-Tyr 15. D-Leu 16. D-Ile

seen, a good resolution has been achieved for various D-, L- amino acid pairs with a good relative retention values approaching 4.3 in the case of Ile. These results are better than other result ($\alpha=1.00-1.28$)¹ and better or worse than other result (α : Val; 4.82, Met; 2.41, Leu; 2.56, Phe; 1.94).⁹ The elution orders between D- and L- amino acids were different according to the functionality of the side chain of amino acids (see Table 2). Generally, L- forms elute before D- forms as shown in Figure 3. But the opposite elution order was observed for His, Asn and Asp. It is considered

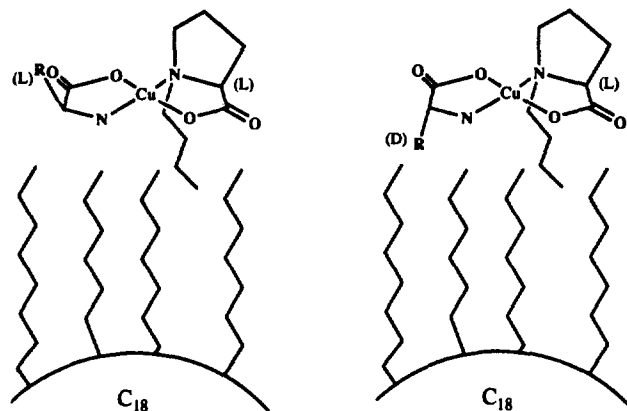


Figure 3. Proposed interaction of the ternary complex containing N-alkyl-L-proline and D- (or L-) amino acid with the alkyl chain of C₁₈ column.

that in the hydrophobic reversed phase sorbents the polar side chain of His, Asn and Asp would tend to be oriented towards the polar mobile phase but not toward the hydrophobic stationary phase as shown in Figure 4. This behaviour indicates that the retention can be illustrated on the basis of the hydrophobic interaction of the ternary complex. At the pH of the maximum enantioselectivity of the amino acid enantiomers, the elution order of the free amino acid enantiomers is generally reversed in contrast with that of the precolumn derivatized amino acid enantiomers.³¹⁻³⁴ This behaviour indicates that the derivatization of amino acids greatly affect the chiral recognition for the enantiomeric separa-

Table 2. Representative Capacity Factor (k_L' and k_D'), Enantioselectivity (α) and Resolution (R_s) for Free Amino Acids

Amino acid	Abbrev.	Structural formula	k_L'	k_D'	α	R_s
Alanine	Ala	<chem>CH3CH(NH2)COOH</chem>	0.47	0.75	1.60	0.50
Valine	Val	<chem>(CH3)2CHCH(NH2)COOH</chem>	1.52	5.28	3.47	5.70
Leucine	Leu	<chem>(CH3)2CHCH2CH(NH2)COOH</chem>	5.14	15.09	2.95	6.63
Isoleucine	Ile	<chem>CH3CH2CH(CH3)CH(NH2)COOH</chem>	4.04	17.31	4.29	9.22
Phenylalanine	Phe	<chem>C6H5CH2CH(NH2)COOH</chem>	20.30	43.23	2.13	4.22
Tryptophan	Trp	<chem>c1ccc2c(c1)c(c[nH]2)C(CCN)C(=O)O</chem>	41.28	94.21	2.28	3.53
Tyrosine	Tyr	<chem>HOC6H4CH2CH(NH2)COOH</chem>	6.14	12.81	2.09	5.09
Lysine	Lys	<chem>H2N(CH2)4CH(NH2)COOH</chem>	0.05	0.09	1.80	0.11
Arginine	Arg	<chem>H2NC(NH)NH(CH2)3CH(NH2)COOH</chem>	0.23	0.41	1.78	0.58
Histidine	His	<chem>NC1=NC=NC(=C1)C(CCN)C(=O)O</chem>	0.84	0.31	0.37 (2.71)	1.06
Asparagine	Asn	<chem>H2NCOCH2CH(NH2)COOH</chem>	0.93	0.29	0.31	2.07
Glutamine	Gln	<chem>H2NCO(CH2)2CH(NH2)COOH</chem>	0.46	0.68	1.48	0.58
Aspartic acid	Asp	<chem>HOOCCH2CH(NH2)COOH</chem>	0.92	0.89	0.97	0.83
Glutamic acid	Glu	<chem>HOOC(CH2)2CH(NH2)COOH</chem>	1.35	2.43	1.80	1.86
Methionine	Met	<chem>CH3S(CH2)2CH(NH2)COOH</chem>	3.28	7.08	2.16	4.32
Serine	Ser	<chem>HOCH2CH(NH2)COOH</chem>	0.36	0.44	1.22	0.17
Threonine	Thr	<chem>CH3CH(OH)CH(NH2)COOH</chem>	0.45	0.53	1.18	0.21

Column: 15×0.46 cm, $3 \mu\text{m}$ (Spherisorb ODS-2), Mobile phase: 3.25×10^{-3} M Cu(N-pentyl-L-proline)₂, 10^{-2} M borate buffer and 10^{-3} M pyridine in aqueous solution at pH: 5.5. Flow rate of mobile phase: 1.0 ml/min. Flow rate of OPA solution: 0.5 ml/min. $\alpha = k_D'/k_L'$

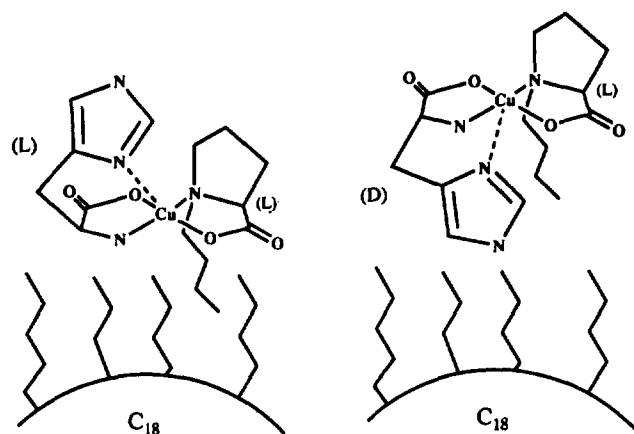


Figure 4. Interaction of the ternary complex containing N-alkyl-L-proline and D- (or L-) His with the alkyl chain of C_{18} column.

Table 3. Capacity Factor (k') and Enantioselectivity (α) on Complex Concentration and Addition of Acetonitrile at pH 6.5

Free-AA		1.625×10^{-3} M		3.25×10^{-3} M		3.25×10^{-3} M	
		100% H_2O		100% H_2O		5% ACN	
		k'	α	k'	α	k'	α
Ala	D	3.10	1.16	2.12	1.44	0.79	1.20
	L	2.68		1.47		0.66	
Val	D	15.71	2.88	9.70	1.95	4.24	3.01
	L	5.46		4.98		1.41	
Leu	D	54.59	3.57	26.92	2.71	10.26	2.60
	L	15.30		9.95		4.14	
Ile	D	46.76	2.96	24.05	3.06	11.96	3.28
	L	15.81		7.85		3.65	
Tyr	D	43.45	2.11	22.09	1.95	6.58	2.12
	L	20.55		11.33		3.10	
His	D	3.11	0.69	1.69	0.70	0.61	0.62
	L	4.51		2.40		0.98	
Asn	D	2.19	0.66	2.92	0.72	0.50	0.78
	L	3.33		4.04		0.64	
Glu	D	3.55	1.25	3.80	1.15	1.50	1.46
	L	2.85		3.32		1.03	
Met	D	25.09	1.76	12.82	1.79	5.55	1.98
	L	14.30		7.17		2.80	
Thr	D	3.78	1.08	2.15	0.99	0.67	1.10
	L	3.49		2.18		0.61	

The experimental conditions are the same as in Table 2.

tion.

The separation of the optical isomers is dependent on the concentration of the $Cu(II)-(N\text{-alkyl-L-proline})_2$ chelate added as shown in Table 3. The capacity factors of the free amino acid enantiomers decrease as the concentration of the chiral chelate additive increases. This behaviour is opposite to that of the derivatized amino acid enantiomers. In the case of the derivatized amino acid enantiomers, the capacity factor increases with increase in the concentration of chiral chelate additive, which means the ternary complex is more hydrophobic than the derivatized amino acids. In the case of the

Table 4. Capacity Factor (k') and Enantioselectivity (α) as a Function of pH

Free-AA		4.5		5.5		6.5	
		k'	α	k'	α	k'	α
Ala	D	0.74	2.62	0.65	1.51	0.79	1.20
	L	0.38		0.43		0.66	
Val	D	2.57	3.47	3.29	2.81	4.24	3.01
	L	0.74		1.17		1.41	
Leu	D	5.45	2.46	8.51	2.61	10.26	2.60
	L	2.22		3.26		4.14	
Ile	D	6.89	3.85	9.89	3.47	11.96	3.28
	L	1.79		2.80		3.65	
Tyr	D	4.16	1.87	5.27	2.09	6.58	2.12
	L	2.22		2.52		3.10	
His	D	0.26	0.67	0.42	0.58	0.61	0.62
	L	0.39		0.73		0.98	
Glu	D	1.16	1.32	1.48	1.48	1.50	1.46
	L	0.88		1.00		1.03	
Met	D	3.05	2.02	4.12	1.96	5.55	1.98
	L	1.51		2.10		2.80	

Mobile phase: acetonitrile/water of 5/95 (v/v). The other experimental conditions are the same as in Table 2.

free amino acids, a decrease of the capacity factor with increase in the chelate concentration indicates that the amino acids, being more hydrophobic than the ternary complex, is more interacted with the stationary phase and so the ternary complex is more hydrophilic. The enantioselectivity is nearly unaffected with change in the concentration of the chiral chelate. This behaviour indicates that although the chiral recognition increases by increase in the ternary complex concentration, the shorter retention decreases the enantioselectivity.

Resolution of the amino acid enantiomers is also dependent on the composition of acetonitrile in the mobile phase as shown in Table 3. It shows that as acetonitrile is added to the mobile phase, the less is the retention of the isomers, as expected in the reversed phase LC. The enantioselectivity shows no definite change with the small addition of acetonitrile. It seems that this behaviour comes from increase in column efficiency.

The capacity factor and enantioselectivity of free amino acid enantiomers varied with the pH of the mobile phase as shown in Table 4. The capacity factors of the free amino acid enantiomers increase with the increase of pH of the mobile phase. This behaviour is different to that of the derivatized amino acid enantiomers. The pH of the eluent governs the dissociation of the amino acid and consequently controls their coordination ability with copper(II) ions. Copper (II) ion is more favoured in a higher pH solution. In the pH range studied, the higher is pH of the eluent, the more retention time is. Since the ternary complex is more hydrophilic than the free amino acids, the free amino acid enantiomers are retained longer with the increase of pH. This retention behaviour is different from that of the derivatized amino acids but it can be explained as the case of Table 3. The enantioselectivity shows no significant difference with

Table 5. Capacity Factor (k') and Enantioselectivity (α) on the Type of Ligand

Free-AA		OCTYL		PENTYL		PROPYL	
		k'	α	k'	α	k'	α
Ala	D	0.34	1.03	2.12	1.44	1.37	1.16
	L	0.33		1.47		1.18	
Val	D	1.34	1.52	9.70	1.95	8.46	1.84
	L	0.88		4.98		4.60	
Leu	D	3.10	1.82	26.92	2.71	23.87	1.94
	L	1.70		9.95		12.23	
Ile	D	3.12	2.35	24.05	3.06	25.88	2.16
	L	1.33		7.85		11.99	
Tyr	D	6.89	1.55	22.09	1.95	16.06	1.53
	L	4.46		11.33		10.51	
His	D	0.11	0.28	1.69	0.70	1.12	0.80
	L	0.39		2.40		1.40	
Glu	D	0.45	0.98	3.80	1.15	3.17	1.27
	L	0.46		3.32		2.49	
Met	D	2.05	2.16	12.82	1.79	11.23	1.45
	L	0.95		7.17		7.76	

pH: 6.5. The other experimental conditions are the same as Table 2.

the change of pH. It was found that the optimal pH range is between 4.5 and 6.5 under the condition of separation employed in this study.

The separation of free amino acid enantiomers is also dependent on the structure of the chiral ligand used as shown in Table 5. In general, the bulkier is the ligand, the more the amino acid is retained. In the case of N-pentyl-L-proline, the free amino acids are more retained than the case of N-propyl-L-proline. It is clear that N-pentyl-L-proline is more hydrophobic than N-propyl-L-proline. The ternary complex of N-pentyl-L-proline is more retained than that of N-propyl-L-proline. However, in the case of N-octyl-L-proline, free amino acids is less retained than that of N-pentyl-L-proline. This behaviour appears due to the fact that the ligand exchange reaction between Cu(II)-(N-octyl-L-proline)₂ and free amino acids is difficult due to the steric hindrance of the long octyl group. This steric effect hinders the ligand exchange reaction for free amino acids to take N-octyl-L-proline ligand in the binary complex away from the chelate plane. The binary complex having the long octyl chain also change the hydrophobic stationary phase to the hydrophilic by coating. Enantioselectivity shows no difference or only a little increase with the increase of the alkyl chain length of ligands.

It is well known that the bidentate ligand such as amino acids forms a chelate in 2 : 1 stoichiometric ratio with copper (II). The copper chelate is well known to have a geometry of square planar and has a *trans* configuration.³¹⁻³⁴ The mechanism of the enantiomeric resolution of the free amino acids is considered as follows as explained in the other works.³¹⁻³⁴

It is assumed that the optical isomers of free amino acids react with the binary copper(II) chelate via S_N2 mechanism to form ternary complexes.³¹⁻³⁴

First, the formation constant of the ligand exchange reaction for one enantiomer can be different from that for the other. It is believed that this process of the ligand exchange reaction affect the enantioselectivity due to the difference of the formation constants between the enantiomeric pairs. It is likely that the difference of the formation constants stems from the steric effect of the ligand exchange reaction (mainly alkyl group of the ligands).

Second, it is also believed that the hydrophobic interaction between the diastereomers formed and the C₁₈ phase is responsible for the enantiomeric resolution of free amino acid. As shown in Figure 3, the hydrophobic interaction the sorbent surface and the diastereomers will be different due to the different structure. The ternary complex of D- isomer is more stabilized than the L- isomer and thus retained longer. It is also obvious why His, Asn and Asp show an opposite elution order compared with the other amino acids as shown in Figure 4. The hydrophilic groups of the enantiomers in the ternary complexes seems to have a repulsive interaction with the C₁₈, reversed phase.

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Enantiomeric Separation of Amino Acids Using N-alkyl-L-proline Coated Stationary Phase

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Enantiomeric separation of underivatized amino acids using N-alkyl-L-proline (octyl, dodecyl or hexadecyl) coated HPLC has been accomplished. The anchoring N-alkyl groups of L-proline provides a permanent adsorption of the resolving chiral agent on the hydrophobic interface layer of a reversed phase. The factors controlling retention and enantioselectivity such as the Cu(II) concentration, pH of the eluent, the type and concentration of organic modifier in the hydroorganic eluent, and extent of coating were examined. The elution orders between D and L- amino acids were consistent, L-forms eluting first, except histidine and asparagine. The extremely high enantioselectivity (α up to 13 for proline) is observed. The retention mechanism for the chiral separation can be illustrated by a complexation and hydrophobic interaction.

Introduction

The separation of racemic amino acids by use of a liquid chromatography has been of great interest in recent years. The prevailing approach is the ligand exchange chromatography based on the formation of diastereomeric complexes between a metal ion and amino acids. Using either the chiral bonded phase or the chiral additive, a high chiral recognition is achieved for a number of D,L-amino acids (free or derivatized) with optically active metal chelate system. Chiral modification of commercially available high performance liquid chromatographic columns by adsorption of an appropriate chiral ligand was investigated in several laboratories.¹⁻⁴ One of these coated CSPs was prepared by Bernauer *et al.* very early. The authors mentioned above prepared ion exchange resins, Dowex 1×2 saturated with optically active anionic complexes and used them as packings in chromatography of racemates.^{5,6} Besides the practical results, these papers are also of interest in the other respect: it has been demonstrated that an adsorptional modification of a nonchiral sorbent with a chiral ligand or complex is a promising way for obtaining chiral packings.

Chiral modification of commercially available high perform-

ance liquid chromatographic columns by adsorption of appropriate chiral ligands combines important advantages of the two approaches (CMPA and CSP) in resolving racemates by means of a ligand exchange chromatography.⁷⁻¹⁴ These advantages are the possibility of using available chromatographic sorbents and applying desired chiral coating agents; the high selectivity of chiral phase system; the unique possibility of eluting the modifier, thus regenerating both the column and the resolving agent; and the possibility of preparative resolutions because of the absence of disturbing organic contaminants in the eluted fractions.

N-Alkyl derivatives of L-hydroxyproline have been used to modify reversed phase column, Lichrosorb RP-18.² The anchoring N-alkyl groups (*n*-C₇H₁₅-, *n*-C₁₀H₂₁- or *n*-C₁₆H₃₃-) of L-hydroxyproline provided a permanent adsorption of the resolving agent on the hydrophobic interface layer of the reversed phase packing material. N-Alkyl-L-histidine also has been used as a resolving agent.³ Kimura *et al.*¹⁵ applied this procedure to the preparation of crown ether coated packings. The very simple technique of modifying column packings *via* adsorption of chiral ligands can be easily extended to the various new systems. Even L-phenylalanine was found¹⁶ to adsorb from aqueous solutions onto a reversed phase