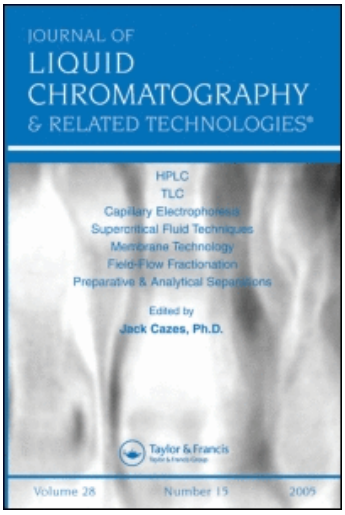

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RESOLUTION OF AMINO ACID ENANTIOMERS BY LIGAND EXCHANGE CHROMATOGRAPHY ON A NEW CHIRAL PACKING

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ABSTRACT

Resolution of α–amino acid enantiomers by ligand exchange chromatography on a new chiral packing, which the chiral ligands were linked onto a hydrophilic polymeric matrix through long spacers, was studied. The packing had high enantioselectivity for most of the amino acids tested. The mechanism of the resolution was discussed. The semi– preparative scale resolution was made.

INTRODUCTION

Chiral ligand exchange chromatography has been extensively adopted in the resolution of amino acid enantiomers. One of its most outstanding advantages is that derivatization of the solute is not necessary. Many applications have illustrated that high enantioselectivity and great separation power are inherent in this method[1–10]. The

probable structures of various types of complexes and the correlation of these structures with the observed enantioselectivities have been the topics of extensive investigations.

The chiral ligand exchange chromatography has been performed by two modes: (i) the chiral ligands are added into the mobile phase (the chiral mobile phase), and (ii) the chiral ligands are immobilized onto the support (the chiral stationary phase). For the first mode, high resolution factors have often been attained. However, the loss of the chiral ligands during the chromatographic process is unavoidable, and most chiral ligands shows intensive UV absorption, which defies the conventional detection. The second mode has no such disadvantages. It is easily applied on preparative purpose. The efficiency of the second mode, however, is generally low because of the slowness of the ligand exchange rate[11].

In order to improve the efficiency of the ligand exchange chromatography on the chiral stationary phase, we have synthesized a chiral polymer [12,13]. In this paper, we have studied thoroughly the resolution of α–amino acid enantiomers on the polymer loaded with Cu(II) ions.

EXPERIMENTAL

Preparation of the Packing

The preparation of the chiral polymer in four steps was described in previous papers[12,13]. The content of the chiral ligand, L–proline, was 1.76 mmole / g of dry polymer. The polymer was shaken in 0.5 M $Cu(OAc)_2$ solution for 12 hours, and then washed with water. A glass column (25 × 0.57 cm, I.D.) was packed with the Cu(II)–coordinated polymer by slurry method. The height of the stationary phase was 20 cm.

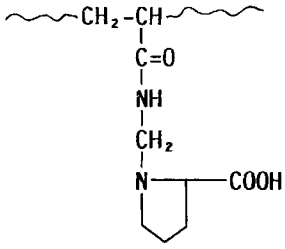
Resolution of Amino Acid Enantiomers

The DL–amino acids were introduced to the top of the column. The elution at the flow rate of 20 ml / h was carried out at room temperature. The eluent was detected with a ZW–II UV Detector (manufactured in Beijing Institute of New Technology Application) at 254 nm.

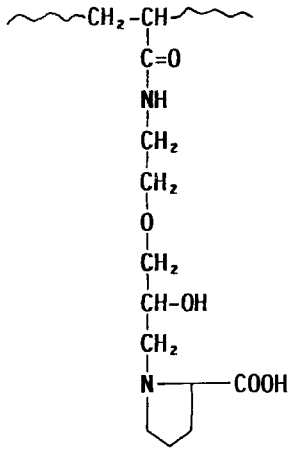
RESULTS AND DISCUSSION

Nature of the Chiral Polymer

The low efficiency of the ligand exchange chromatography (employing the chiral stationary phase) is mainly due to the slow ligand exchange rate in the chromatographic process. Lefebvre et al.[7] synthesized a chiral polymer with hydrophilic polyacrylamide matrix (structure I) , with which the efficiency of the resolution of amino acid enantiomers was markedly improved. Many underivatized racemic α – amino acids were resolved on the polymer loaded with Cu(II) ions by elution with water. However, we found that the methylene bridges between the chiral ligand (L–proline) and the polymeric matrix were not stable under acidic and basic conditions. For example, when the column packed with the Cu (II)–coordinated polymer I was eluted with 0.1 M NH_3 or 0.1 M $(NH_4)_2CO_3$ aqueous solutions, which are often used as eluents for the resolution of amino acid enantiomers by ligand exchange chromatogaphy, the chiral ligand, L–proline, was eluted out gradually. Therefore, the practical application of the chiral polymer is limited. We synthesized a new chiral polymer, structure II , which possesses a hydrophilic



I



II

Table 1 Resolution of amino acid enantiomers. The eluents contained 1×10^{-4} M $Cu(OAc)_2$, $\alpha = \frac{k'_L}{k'_D}$; Other conditions as given in Figure 1.

eluent / solute	0.1M $(NH_4)_2CO_3$			0.2M $(NH_4)_2CO_3$			0.1M NH_3 / 0.1M NH_4Cl		
	k'_D	k'_L	α	k'_D	k'_L	α	k'_D	k'_L	α
histidine				3.65	45.92	12.7			
tryptophan				5.23	19.23	3.68			
tyrosine	9.45	36.63	3.66	1.69	5.77	3.41			
phenylalanine	4.35	11.24	2.58	1.15	2.54	2.21	23.31	57.46	2.47
phenylserine				1.34	2.48	1.85			
proline	6.91	3.30	0.48	1.03	0.62	0.60	17.35	8.95	0.50
serine	3.91	6.46	1.65				16.5	28.5	1.73
threonine	4.31	6.54	1.51				15.2	23.4	1.54
valine	4.27	6.47	1.52	0.74	1.05	1.42	12.2	18.9	1.55
isoleucine	4.91	6.64	1.35				14.5	22.1	1.52
leucine	6.37		1.00	1.15		1.00	22.63		1.00
α–aminobutyric acid	3.60		1.00	0.85		1.00			
alanine	3.56		1.00	0.71	0.89	1.25			
methionine	4.52		1.00	1.03		1.00			
lysine	1.63		1.00						
aspartic acid				0.54	1.11	2.05			
glutamic acid	0.58		1.00	0.31		1.00			

polyacrylamide matrix and a long spacer that may increase the ligand exchange rate. When polymer Ⅱ was soaked in 0.1 M HCl, 0.1 M NaOH or 1 M NH_3 aqueous solutions, respectively, at room temperature for one week, the nitrogen content and Cu(Ⅱ) capacity of the polymer didn't change, indicating that polymer Ⅱ is stable under moderately acidic or basic conditions.

Resolution of Amino Acid Enantiomers

Figure 1 and Table 1 summarize the resolution of amino acid enantiomers on the column packed with the Cu (Ⅱ) – coordinated

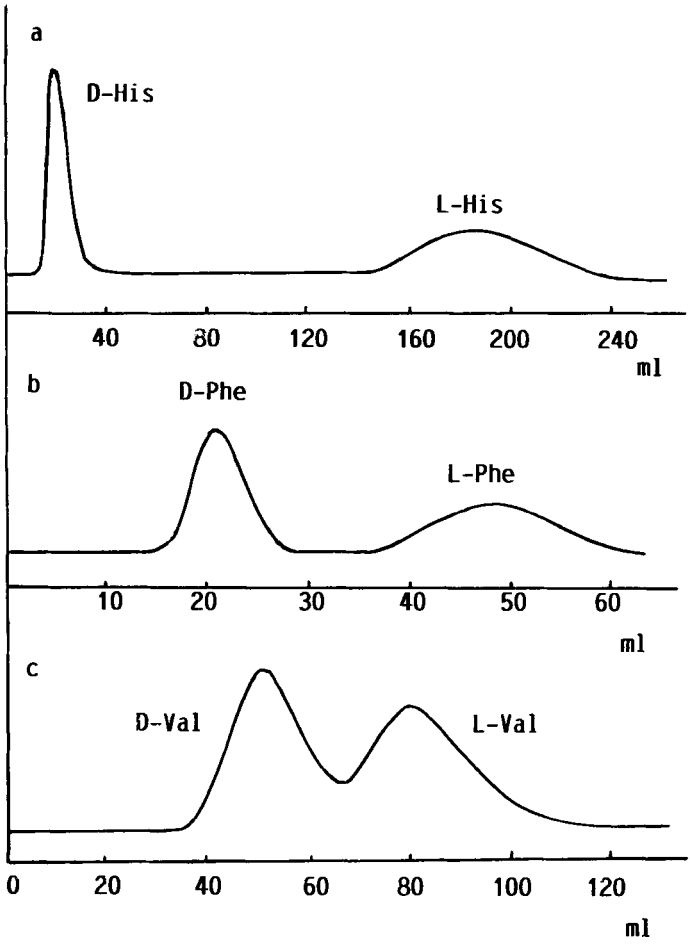
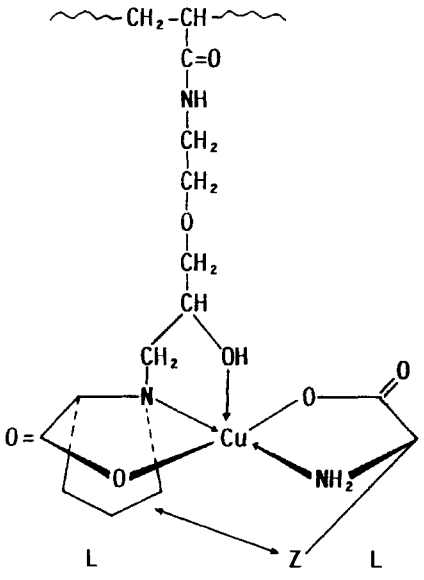


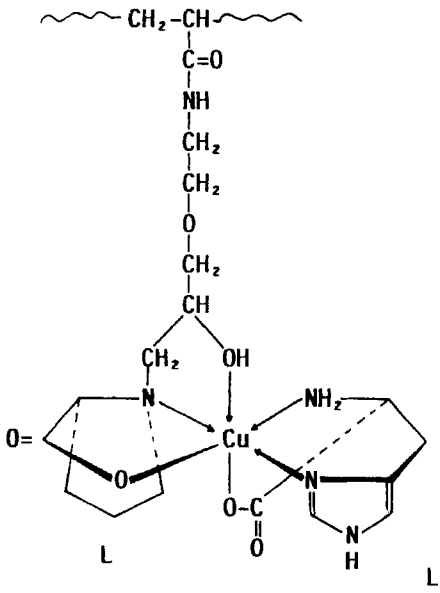
Figure 1: Chromatograms of amino acid enantiomers on Cu(II)–coordinated polymer II.
Column: 20 × 0.57 cm I.D.; Flow rate: 20 ml / h; Eluent: a, 0.2 M $(NH_4)_2CO_3$–1 × 10^{-4} M $Cu(OAc)_2$; b, 0.1 M $(NH_4)_2CO_3$–1 × 10^{-4} M $Cu(OAc)_2$; c, 0.1 M NH_3 / 0.1 M NH_4Cl–1 × 10^{-4} M $Cu(OAc)_2$; Amount of the solute: 2 mg.

polymer Ⅱ. As can be seen from the figure and the table, the packing has high enantioselectivities for many of the racemic α – amino acids, especially for aromatic amino acids. The L – enantiomers are more strongly retained for the racemates tested except proline, for which the reverse elution order is observed.

Structure Ⅲ shows the probable structure of the complex formed between the Cu(Ⅱ) ion, grafted L – proline and L–enantiomer. The α–amino groups and α–carboxyl groups of both the grafted L–proline and the L–enantiomer coordinate with the central Cu(Ⅱ) ion in a square plane, and the hydroxyl group on the polymer coordinates in axial position. It can be seen that the complex may be stabilized by hydrophobic interaction between the side chain of the grafted L–proline and that of the L–enantiomer. In contrast, the complex formed with the D–enantiomer suffers from steric hindrance between the hydroxyl group on the polymer and the side chain of the



III



IV

D–enantiomer. Therefore the D–isomer is eluted first and the L–isomer next.

The high enantioselectivity for the aromatic amino acids on the packing is observed. It may be due to the fact that the side chain of the L–enantiomer interacts with the central Cu(II) ion in the axial position, thus stabilizing the complex.The explanation is supported by studies of the formation constants and enthalpy changes[14,15], and circular dichroism (CD)[16]. In contrast, there is no such interaction for the D–isomer because the hydroxyl group on the polymer has occupied the upper axial position.

The enantioselectivities for serine and threonine are higher than those for the corresponding aliphatic amino acids, alanine and α–aminobutyric acid, and even higher than those for the other aliphatic amino acids, valine, leucine and isoleucine. It is difficult to explain by the hydrophobic interaction and steric hindrance mechanism mentioned above. We suppose that the hydroxyl groups of the L–isomers of serine and threonine coordinate to the central Cu(II) ion in the axial position, which stabilized the complex formed. The assumption is supported by the fact that although the coordination affinities of serine and threonine for Cu(II) ion are lower than those of the aliphatic amino acids[17], the retentions of the L–isomers of the formers are still stronger than or close to those of the latters.

The Cu(II)–loaded polymer II has much higher enantioselectivity for DL–histidine than for the other amino acid enantiomers. Freeman and his co–workers[18,19] reported the structure of ternary complex L–histidine–Cu(II)–L–threonine in which L–histidine was tridentate involving N–amino and N–imidazole coordination in a square plane around the Cu(II) ion and the carboxyl oxygen in the axial position. If the structure of the complex formed on the polymer is similar to that found in the ternary complex[19], i.e. with the α–amino groups *cis*, then the structure IV may be the structure of the complex formed on the polymer with L–histidine. The complex is stabilized by the coordination of the carboxyl group to the central Cu(II) ion in the axial position. The complex formed with D–histidine, however, is much less stable because the upper axial position of the central Cu(II) ion has been blocked by the hydroxyl group on the polymer. Therefore, the packing has high enantioselectivity for DL–histidine.

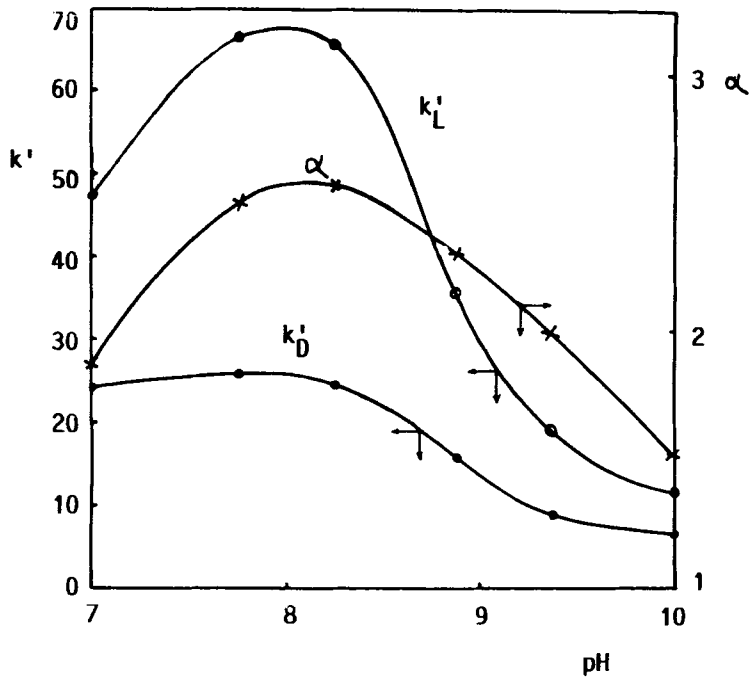


Figure 2: Dependence of the capacity factor, k′, and the selectivity factor, α, on the pH of eluent.
Solute: DL–phenylalanine; Eluent: 0.2 M NH_3 / NH_4Cl; Other conditions as given in Figure 1.

Effect of pH of the Eluent on the Resolution

Since both the grafted chiral ligand and the solute to be resolved contain acidic and basic groups, the pH of the eluent influences the retentions and selectivity factors. Figure 2 shows the effect of the pH values of the eluent on the resolution of DL–phenylalanine using NH_3 / NH_4Cl aqueous solution as the eluent. The pH values were adjusted by changing the ratios of the concentration of NH_3 to that of NH_4Cl while the sum of the concentrations of ammonia and ammonium chloride remains unchanged, i.e. $[NH_3]+[NH_4Cl] = 0.2$ M. The capacity factor,k′, of the L–isomer is more strongly influenced by the pH of the eluent than that of the D–isomer. The maximum value of the enantioselectivity factor, α, is observed at pH 8.1.

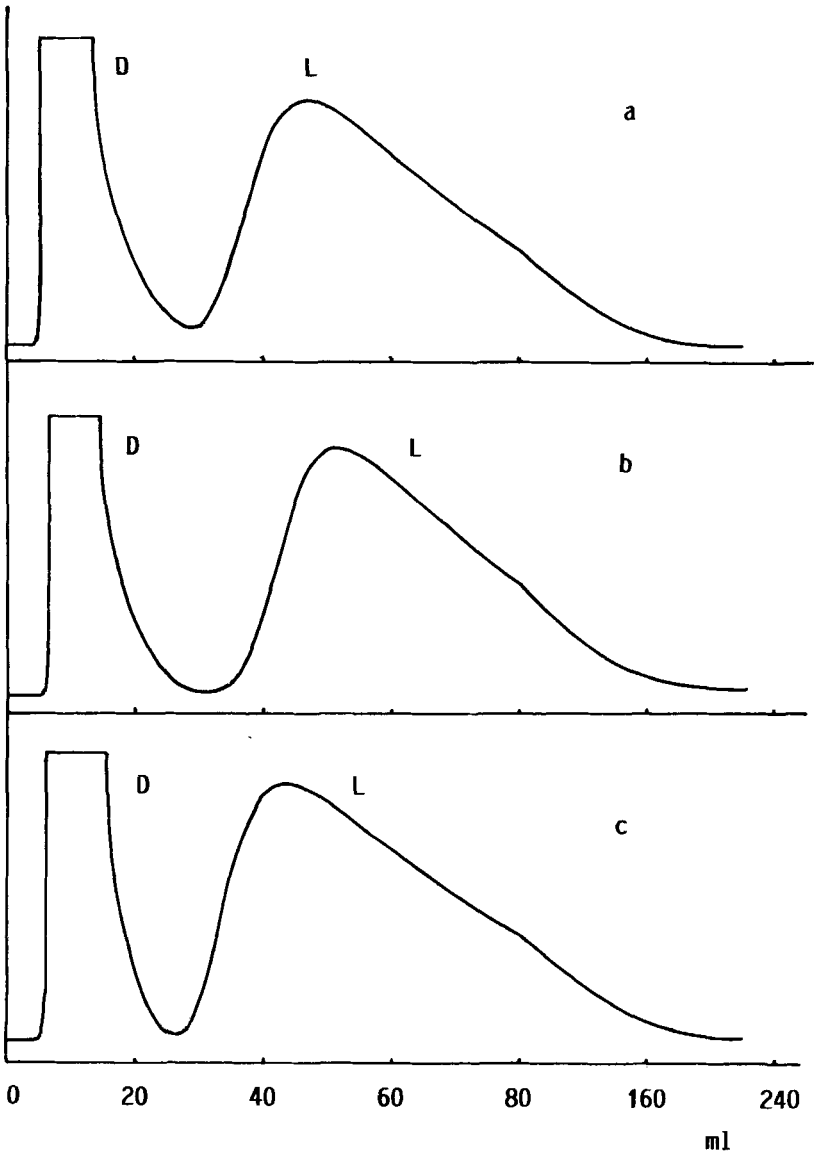


Figure 3: Chromatograms of semi–preparative resolution of DL histidine.
Eluent: 0.2 M $(NH_4)_2CO_3$–1 × 10^{-4} M $Cu(OAc)_2$: Solute: a, 40 mg of DL–histinine; b, 40 mg of DL–histidine ([DL–His] / [Cu(II)] = 2 / 1); c, 50 mg of DL–histidine ([DL–His] / [Cu(II)] = 2 / 1); Other conditions as given in Figure 1.

Semi–preparative Resolution of DL–histidine

The resolution of amino acid enantiomers by the chiral ligand exchange chromatography has the advantage of simplicity. However, the practical application is limited because only a small amount of sample can be resolved in each run. Our packing has high enantioselectivities for aromatic amino acids, especially for histidine, and thus has high resolving capacities. A semi–preparative resolution of DL–histidine has been studied.

As discussed above, histidine is a tridentate ligand. It has high coordination affinity for Cu(II) ion[17]. When 40 mg of DL–histidine was introduced into the column (20 × 0.57 cm I.D.), the colour of upper resins about 1.5 cm high changed from blue (the colour of the complexed Cu(II) ions) to yellow (the colour of polymer II), indicating that the following ligand exchange may occur at the beginning of the chromatography:

$$\overline{R}Cu(NH_3)_2 + 2\ His \longrightarrow \overline{R} + 2\ NH_3 + Cu(His)_2$$

where $\overline{R}$ represents polymer II. If the DL–histidine first coordinated with Cu(II) ions in the ratio of 2 / 1 (mole ratio) and was then introduced into the column, the selectivity of the column increased, as shown in Figure 3. Moreover the solubility of DL–amino acids to be resolved increases with the addition of Cu(II) ions. It is favourable to the solute that has low solubility in water. Figure 3c shows that 50 mg of DL–histidine has been completely resolved on the column. The preparative resolution on a larger scale is in progress.

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