Journal of Chromatography, 217 (1981) 539–543
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,958

LIGAND-EXCHANGE CHROMATOGRAPHY OF AMINO ACID RACEMATES ON SEPARON GELS CONTAINING L-PROLINE OR L-HYDROXYPROLINE GROUPINGS*

I. A. YAMSKOV, B. B. BEREZIN* and V. A. DAVANKOV
Institute of Organo-Element Compounds, Academy of Sciences, Moscow (U.S.S.R.)
and
Yu. A. ZOLOTAREV, I. N. DOSTAVALOV and N. F. MYASOEDOV
Institute of Molecular Genetics, Academy of Sciences, Moscow (U.S.S.R.)

SUMMARY

Asymmetric resins chelating copper(II) ions to L-proline or L-hydroxyproline ligands fixed in macroporous methacrylate copolymers were synthesized. The sorbents were used for the ligand-exchange chromatography of amino acid racemates and showed high enantioselectivities by retaining preferentially the L-isomers of the amino acids tested (except proline).

INTRODUCTION

Much of the success in developing ligand-exchange chromatography for the resolution of racemates (for a review, see ref. 1) is due to the use of the heterocyclic amino acids L-proline and L-hydroxyproline as the resolving chiral ligands. The first quantitative resolution of a racemic amino acid was achieved with the help of a copper(II)-loaded L-proline-incorporated polystyrene resin[2]. The L-hydroxyproline containing analogue has been used for the rapid enantiomeric analysis of different amino acids[3] and for the production of tritium-labelled optically active preparations[4]. N-Alkyl derivatives of L-hydroxyproline[5] and alkylamides of L-proline[6] are excellent chiral modifiers for commercially available reversed-phase silica gels. Bis(L-prolinato)copper made it possible to develop the "chiral eluent" mode[7,8] of ligand-exchange racemate resolution. Promising sorbents were obtained by covalent fixation of L-proline or L-hydroxyproline on polyacrylamide beads[9] and, recently, on a silica gel surface[10].

While always producing highly enantioselective systems, L-proline–copper complexes display a greater affinity towards L- or D-enantiomers of amino acids, depending on the mode of fixation of the resolving L-proline ligand to the stationary phase. This points to the important role of the spacer (between the fixed ligand itself and the sorbent matrix) in the stereochemistry of ternary sorption complexes. The

* Part XVII of the series "Ligand-exchange chromatography of racemates".

0021-9673/81/0000–0000/$02.50 © 1981 Elsevier Scientific Publishing Company

nature of the matrix should play a subordinate role if is not involved in additional interactions with any components of the sorption complex.

To confirm this suggestion, polymethacrylate-type resins I and II were synthesized by reaction of glycidyl groups of the polymer matrix with L-proline and L-hydroxyproline. These resins were compared with sorbents III and IV of Gübitz *et al.*[10], which have a similar structure of the fixed ligands, prepared by bonding 3-glycidoxypropyltrimethoxysilane to silica, followed by reaction with amino acids.

Y–O–CH_2–CH(OH)–CH_2–N (pyrrolidine ring bearing X and COOH)

I X = OH Y = ~CH_2–$CH(CH_3)$~ with –C(=O)– attached

II X = H Y = ~CH_2–$CH(CH_3)$~ with –C(=O)–O–CH_2–CH_2 attached

III X = H Y = >Si– with CH_2–CH_2–CH_2– attached

IV X = OH

EXPERIMENTAL

Sorbent I

Macroporous poly(2,3-epoxypropyl methacrylate) (G-Gel-60) (13–17 μm; specific surface area 55 m^2/g) was used as the starting material.

In a three-necked vessel were placed 3.3 g (18.1 mmole) of L-hydroxyproline methyl ester hydrochloride, 1.8 g (18.2 mmole) of potassium hydrogen carbonate and 9 ml of methanol and the mixture was stirred for 1 h at 50°C. Then 3.2 g of G-Gel-60 in 5 ml of methanol were added to the reaction mixture, which was heated at 55°C for 2 h. Hydrolysis of the ester groups introduced into the sorbent was carried out in a 0.1 *M* solution of copper(II) sulphate in 0.5 *M* aqueous ammonia for 20 h. After filtration, the copper-containing polymer was rinsed with 0.1 *M* aqueous ammonia and then with 0.1 *M* ammonium carbonate solution containing $2.0 \cdot 10^{-4}$ *M* copper(II) ions.

Sorbent II

L-Proline (0.68 g, 6 mmole) was mixed with Separon H1000 (10–14 μm) (4 g, 2 mmole of epoxy groups) in 28 ml of dioxane in the presence of 2.08 ml of tributylamine and the mixture was heated at 80°C for 10 h. The final product was carefully washed and loaded with copper(II) ions in a 0.1 *M* solution of copper(II) chloride in 0.5 *M* aqueous ammonia. The copper content of the resin was 0.3 mmole/g, which probably corresponds to the content of L-proline fixed ligands.

Chromatography

A Radiochromatograph 2301 liquid chromatograph (U.S.S.R.) equipped with a microcolumn line and a UV detector (250 nm) was used for chromatography with sorbent I with L-hydroxyproline groupings. Glass columns (20 cm × 0.2 cm I.D.) were packed by the ascending slurry technique. The flow-rate was 5 ml/h. The amount of amino acid introduced in the column was 20 μg. Copper-containing ($2 \cdot 10^{-5}$–$1 \cdot 10^{-4}$ *M*) solutions of ammonium carbonate (0.02–0.10 *M*), pH 9.5, were used as the eluent.

A stainless-steel column (20 cm × 0.4 cm I.D.) and a UV detector (250 nm) were used for chromatography with sorbent II with L-proline groupings. The eluents used were 0.1 *M* aqueous ammonia and 0.1 *M* ammonium carbonate solution. The flow-rate was 6.4 ml/h. The amount of amino acid introduced was 0.4 μmole.

RESULTS AND DISCUSSION

The results of the ligand-exchange chromatography of amino acid enantiomers on the copper(II)-loaded chiral organic resins I and II, summarized in Tables I and II, are in good qualitative agreement with the recently published results of Gübitz *et al.*[10] obtained on silica gel sorbents III and IV having similar chiral fixed ligands. All four sorbents retain the L-enantiomers of amino acids more strongly, the only exception (of the racemates tested) being proline, for which the elution of the L-isomer preceded that of the D-isomer. From the quantitative point of view, the agreement between the enantioselectivity values, $\alpha = k'_L/k'_D$, was also acceptable, although for some amino acids (histidine, methionine, valine and proline) better results were obtained in our experiments and in other instances *vice versa*.

TABLE I

CHROMATOGRAPHY OF AMINO ACID ENANTIOMERS ON THE Cu(II) FORM OF SORBENT I CONTAINING GROUPINGS OF L-HYDROXYPROLINE

Eluent A, 0.1 *M* $(NH_4)_2CO_3$ (pH 9.5)–$1 \cdot 10^{-4}$ *M* Cu(II); eluent B, 0.02 *M* $(NH_4)_2CO_3$ (pH 9.5)–$2 \cdot 10^{-5}$ *M* Cu(II).

Amino acid	*Eluent A*			*Eluent B*		
	k'_L	k'_D	$\alpha = k'_L/k'_D$	k'_L	k'_D	$\alpha = k'_L/k'_D$
Proline	0.6	1.4	0.4	2.1	5.5	0.38
Alanine	0.7	0.7	1.0	2.8	2.8	1.0
Valine	1.0	0.8	1.2	4.1	3.7	1.10
Leucine				7.0	7.0	1.0
Phenylalanine	10.8	6.0	1.8			
Tyrosine	13.0	4.6	2.83			
Histidine	6.6	1.4	4.70			
Tryptophan	58.0	27.6	2.12			
Methionine	2.4	2.1	1.14			
Threonine	1.1	0.8	1.4	5.8	4.2	1.38
Arginine	2.4	1.8	1.33			
Lysine				6.4	4.6	1.39
Asparagine				6.1	4.4	1.38
Cysteine	7.8	5.0	1.51			

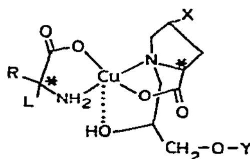
TABLE II

CHROMATOGRAPHY OF AMINO ACID ENANTIOMERS ON THE Cu(II) FORM OF SORBENT II CONTAINING GROUPINGS OF L-PROLINE

Eluent A, 0.1 M NH_3–$1 \cdot 10^{-5}$ M Cu(II); eluent B, 0.1 M $(NH_4)_2CO_3$ (pH 9.5)–$1 \cdot 10^{-4}$ M Cu(II).

Amino acid	*Eluent A*			*Eluent B*		
	k'_D	k'_L	$\alpha = k'_L/k'_D$	k'_D	k'_L	$\alpha = k'_L/k'_D$
Proline		–		0.93	0.53	0.57
Alanine	1.62	1.62	1.0	0.47	0.47	1.0
Valine	1.85	2.21	1.19	0.83	1.17	1.41
Leucine	4.71	4.87	1.03	2.07	2.17	1.05
Threonine	0.51	0.67	1.31	0.39	0.63	1.62
Methionine		–		1.53	2.07	1.35
Histidine	11.9	22.8	1.92	1.05	2.93	2.79
Tyrosine	0.72	1.0	1.39	6.38	11.0	1.72
Phenylalanine	16.1	19.6	1.22	14.6	19.8	1.36

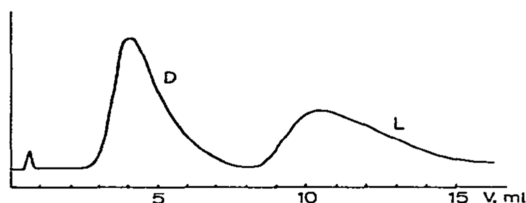
No doubt the stereochemical situation in the ternary sorption complex is governed by the fixed ligand and the neighbouring part of the spacer connecting this ligand with the sorbent matrix. For the stabler diastereomer of the ternary sorption complex, the following structure can be suggested:



The exact character of the inter-ligand interactions and the nature of the enantioselectivity still remain largely unknown. Obviously, much could be learned from an understanding of the exceptional behaviour of proline as the mobile ligand.

As far as the sorbent efficiency is concerned, it must be admitted that the plate number of the columns is low. The HETP is 5 mm for the retained amino acids, but is *ca.* 10 times lower for the unretained sodium acetate. As can be seen from Figs. 1 and 2, the peak resolution is due entirely to the high enantioselectivity values. It should be emphasized, however, that the polymethacrylate matrices used in this work were designed for the chromatography of large molecules and are far from optimal for amino acid molecules. By using a proper matrix, much more efficient chiral sorbents can be synthesized. It is important that the polymethacrylate-type ligand exchangers have the great advantage over their silica gel analogues of possessing excellent chemical stability.

A final comment should be made about the unusually strong dependence of the k' values on the amount of sorbate introduced into the column. On chromatography of 10, 20 and 40 μg of D-phenylalanine on sorbent I, the peak capacity factors were 8.4, 6.0 and 4.8, respectively.



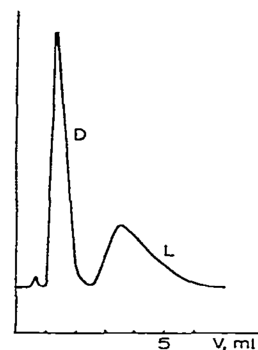


Fig. 1. Chromatography of 8 μg of racemic tyrosine using sorbent I containing L-hydroxyproline groupings. Column: 200 × 2 mm I.D. Eluent: 0.1 *M* $(NH_4)_2CO_3$ (pH 9.5), 5 ml/h.

Fig. 2. Chromatography of 40 μg of racemic histidine. Conditions as in Fig. 1.

ACKNOWLEDGEMENTS

We gratefully acknowledge gifts of samples of Separon H1000 and G-Gel-60 by Drs. J. Čoupek and J. Hřadil (Institute of Macromolecular Chemistry, Prague, Czechoslovakia).

REFERENCES

1 V. A. Davankov, *Advan. Chromatogr.*, 18 (1980) 139.
2 S. V. Rogozhin and V. A. Davankov, *Dokl. Akad. Nauk SSSR*, 192 (1970) 1288; *Chem. Commun.*, (1971) 490.
3 V. A. Davankov, Yu. A. Zolotarev and A. A. Kurganov, *J. Liq. Chromatogr.*, 2 (1979) 1191.
4 N. F. Myasoedov, O. B. Kuznetsova, O. V. Petrenik, V. A. Davankov and Yu. A. Zolotarev, *J. Labelled Compd. Radiopharm.*, 17 (1980) 439.
5 V. A. Davankov, A. S. Bochkov, A. A. Kurganov, P. Roumeliotis and K. K. Unger, *Chromatographia*, 13 (1980) 677.
6 Y. Tapuhi, N. Miller and B. L. Karger, *J. Chromatogr.*, 205 (1981) 325.
7 P. E. Hare and E. Gil-Av, *Science*, 204 (1979) 1226.
8 E. Gil-Av, A. Tishbee and P. E. Hare, *J. Amer. Chem. Soc.*, 102 (1980) 5115.
9 B. Lefebvre, R. Audebert and C. Quivoron, *J. Liq. Chromatogr.*, 1 (1978) 761.
10 G. Gübitz, W. Jellenz and W. Santi, *J. Chromatogr.*, 203 (1981) 377.