Journal of Chromatography, 511 (1990) 115–121
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 421

Ligand-exchange chromatography of α-trifluoromethyl-α-amino acids on chiral sorbents

S. V. GALUSHKO*, I. P. SHISHKINA, V. A. SOLOSHONOK and V. P. KUKHAR
Institute of Bioorganic Chemistry, Academy of Sciences of the Ukrainian SSR, 252660 Kiev 94 (U.S.S.R.)
(First received August 7th, 1989; revised manuscript received February 23rd, 1990)

ABSTRACT

The chromatographic behaviour of some α-trifluoromethyl-α-aminoacids on L-proline- and L-hydroxyproline sorbents was studied. The retention and selectivity parameters of the separation of amino acid enantiomers on the sorbents were determined. The introduction of a CF group led to an increased selectivity in the separation of amino acid enantiomers on a proline sorbent and to a decreased selectivity on a hydroxyproline sorbent.

INTRODUCTION

The bioactivity of synthetic analogues of natural compounds depends primarily on the enantiomer used, so it is important to find optimum conditions for obtaining individual enantiomers and to develop methods for controlling their enantiomeric purity. Fluoroamino acids (FAA) have high biological activity[1], but few studies have been devoted to the enantiomer analysis of FAA. The separation of some FAA by ligand-exchange chromatography has been described[2]. A method for controlling the enantiomeric purity of α-trifluoromethylalanine (α-CF_3Ala) by reversed-phase liquid chromatography with a chiral mobile phase has been developed[3]. No data concerning the ligand-exchange chromatography of α-trifluoromethyl-α-amino acids (α-CF_3AA) on chiral sorbents have been reported.

The aims of this work were to determine the optimum conditions for the ligand-exchange chromatography (LEC) of α-CF_3AA enantiomers and to compare the selctivities of separation of the α-CF_3AA and their natural analogues on L-proline and L-hydroxyproline sorbents.

EXPERIMENTAL

Chromatographic conditions

The experiments were performed on an LKB (Bromma, Sweden) liquid chromatographic system consisting of a Model 2150 high-performance liquid chromatographic (HPLC) pump, a Model 2152 LC controller, a Model 7410 injector,

0021-9673/90/$03.50

a Model 2151 variable-wavelength monitor operated at wavelength 225 nm and a Model 2220 recording integrator. The columns used were (i) a proline column with chiral ProCu=Si100 Polyol (L-Proline covalently bound to Si100 Polyol, 5 μm), 250 × 4.6 mm I.D. (Serva, Heidelberg, F.R.G.), (ii) a hydroxyproline column with Nucleosil Chiral-1 (L-hydroxyproline covalently bound to Nucleosil 120, 5 μm), 250 × 4.0 mm I.D. (Macherey-Nagel, Düren, F.R.G.) and (iii) a Microbe glass column with Chiral ProCu=Si100 Polyol (5 μm), slurry packed, 100 × 1.0 mm I.D. The mobile phase was $0.1 \cdot 10^{-3}$–$5 \cdot 10^{-3}$ *M* copper sulphate solution at a flow-rate of 1.0 ml/min (i, ii) or 0.03 ml/min (iii).

Materials

α-Trifluoromethyl-α-amino acids and villardine were obtained as described[4,5]. Natural amino acids were supplied by Reakhim (Moscow, U.S.S.R.). Copper sulphate and hydrochloric acid (analytical-reagent grade) were used as received. Water was doubly distilled and filtered for HPLC use.

RESULTS AND DISCUSSION

In isocratic LEC the ligand (A) is usually chemically bonded to the surface of a sorbent and the mobile phase contains a constant concentration of metal ions (M). When a certain amount of another ligand (B) is introduced into the mobile phase, a mixed complex $(MAB)_s$ is formed on the surface. The capacity factor of the ligand B can be defined as follows:

$$k' = \varphi \cdot \frac{[MAB]_s}{[B]_m + [MB]_m + \ldots + [MB_n]_m} \tag{1}$$

where φ is the phase ratio and m and s represent the concentrations in the mobile phase and on the sorbent surface, respectively.

The formation of a mixed complex on the surface can be represented by

$$\begin{aligned} &(MA)_s + (B)_m \rightleftharpoons (MAB)_s \\ &K_1[MA]_s[B]_s = [MAB]_s \end{aligned} \tag{2}$$

where K_1 is the equilibrium constant. The concentration of a metal ion in the mobile phase (C_M) is usually much higher than that of the ligand B (C_B), and we can assume that in the mobile phase a complex MB exists:

$$\begin{aligned} &(M)_m + (B)_m \rightleftharpoons (MB)_m \\ &K_2[M]_m[B]_m = [MB]_m \end{aligned} \tag{3}$$

When the complex MB is weakly dissociated, $[MB]_m \gg [B]_m$ and

$$k' = \varphi \cdot \frac{[MAB]_s}{[MB]_m} \tag{4}$$

As $C_M \gg C_B$, it can be assumed that $[M]_m \approx C_M$, and using eqns. 2 and 3, eqn. 4 can be written as follows:

$$k' = \varphi \cdot \frac{K_1[MA]_s}{K_2 C_M}$$

When the ions of metal are in abundance and the complex formed, $(MA)_s$, is strong, almost all the surface ligands are bonded in the complex $(MA)_s$; therefore, the concentration of this complex increases insignificantly with increase in the concentration of M ions in the mobile phase. Taking into account that $[MA]_s \gg [MAB]_s$, one can assume $[MA]_s \approx$ constant, and then

$$K' = \frac{Q}{C_M}$$

where $Q = (K_1[MA]_s)/K_2$. Hence a plot of k' *vs.* $1/C_M$ is a straight line of slope Q passing through the origin. Q is a constant for a given column and ligand B. Plots of k' *vs.* $1/C_M$ for several amino acids are shown in Fig. 1. As can be seen, the deflection from a straight line is observed only with a low concentration of Cu^{2+} ions in the mobile phase (lower than $5 \cdot 10^{-4}$ *M*), *i.e.*, when the condition $C_M \gg C_B$ is not satisfied. The values of Q can be determined when the columns are tested. Knowing the Q values, the capacity factors of enantiomers can be calculated exactly under various conditions of metal ions in the mobile phase.

These experiments have shown that the enantiomers of α-CF_3AA can be successfully separated on chiral sorbents containing proline and hydroxyproline. The efficiency of the columns is so high that the separation is easily achieved even at ambient temperature (Fig. 2). The optimum concentrations of Cu^{2+} ions in the mobile phase are $1 \cdot 10^{-3}$–$5 \cdot 10^{-3}$ *M*. For poorly retained 3,3,3-trifluoroalanine and α-trifluoromethylalanine it is preferable to use a mobile phase with a low Cu^{2+} concentration, whereas for α-CF_3Phe it is necessary to use a mobile phase with a Cu^{2+} concentration not lower than $5 \cdot 10^{-3}$ *M*. The chromatographic behaviour of α-trifluoromethylphenylglycine differs greatly from that of other α-CF_3AA. The retention and selectivity of separation of α-trifluoromethylphenylglycine enantiomers are so great that it is necessary to use a mobile phase with a higher concentration of H^+ ions and the separation should be carried out at 45–55°C. An eluent of composition A–B = 75:25 or 50:50, where A is $5 \cdot 10^{-3}$ *M* $CuSO_4$ containing ethanol (10%, v/v) and B is $5 \cdot 10^{-3}$ *M* $CuSO_4$(pH 3.0; HCl) are optimum for the separation of enantiomers of α-trifluoromethylphenylglycine (Fig. 3). The selectivity of separation (α) is about 3.5, *i.e.*, the conditions are suitable for both enantiomeric analysis and preparative work.

There is a great difference in the retention and selectivity of separation of the amino acid enantiomers on proline and hydroxyproline sorbents (Table I). Si100 Polyol is a polyol derivative containing propylglycerol groups. The surface area of Si100 Polyol (matrix of proline sorbent) is higher than that of Nucleosil Chiral-1 (300 and 200 m^2/g, respectively) and the retention of amino acids on the proline column is higher than that on the hydroxyproline column. The α-CF_3 group exerts a consider-

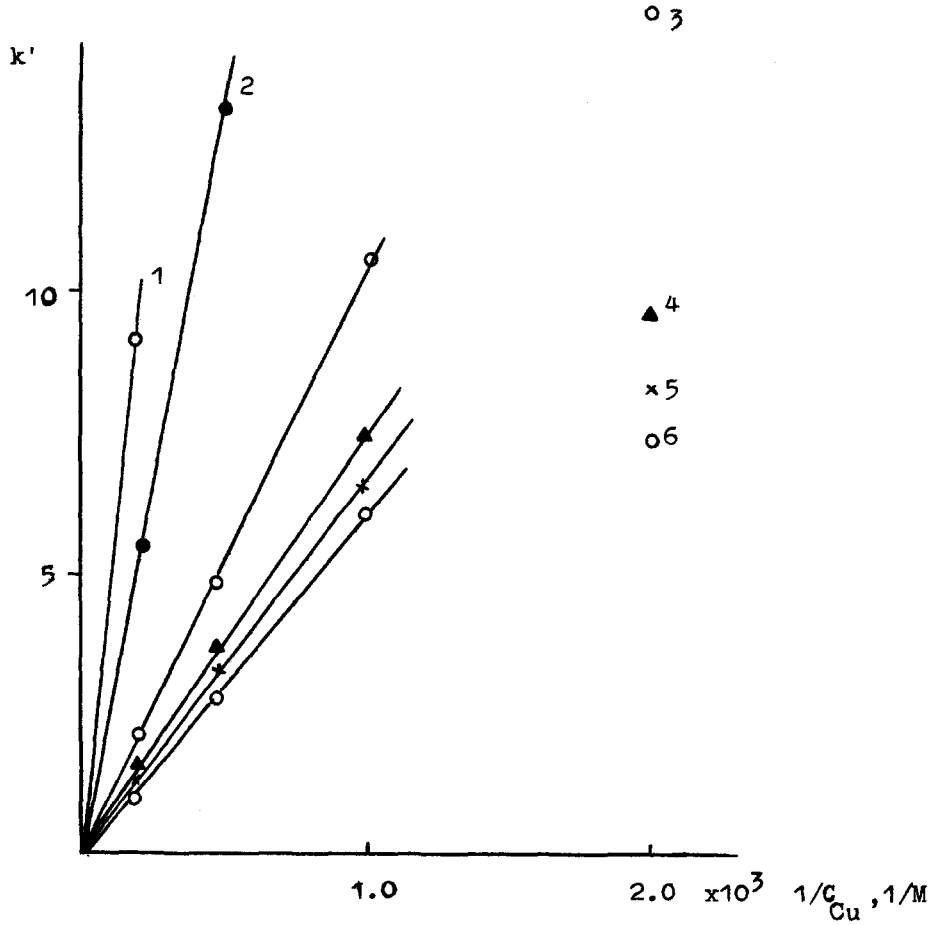

Fig. 1. Effect of $CuSO_4$ concentration on capacity factors: 1 = L-α-CF_3Phe; 2 = D-α-CF_3Phe; 3 = L-α-CF_3Ala; 4 = L-3,3,3-trifluoroalanine; 5 = D-α-CF_3Ala; 6 = D-3,3,3-trifluoroalanine. Microbore glass column (100 × 1.0 mm I.D.) with chiral ProCu=Si100 Polyol, 5 μm; flow-rate, 0.01 ml/min.

able influence on both the retention and the selectivity of the separation of amino acid enantiomers. The retention of α-CF_3AA is much higher than that of natural amino acids. The change in the free energy of sorption when the CF_3 group is introduced is 1.2–2.5 kJ/mol on the proline column and 0.8–4.4 kJ/mol on the hydroxyproline column (Table I). These results imply that the stability of mixed complexes (ProCu B) for α-CF_3AA is higher than that for natural amino acids. The differences in the selectivity of the separation of enantiomers on proline and hydroxyproline columns is considerable (Table II). On the proline column the selectivity of separation of α-CF_3-AA enantiomers is much higher than that of natural amino acids. For example, for Leu, Nle and Ala no separation of enantiomers takes place, whereas for α-CF_3-AA the separation is excellent. The situation is different with the hydroxyproline column; the selectivity of separation of α-CF_3-AA enantiomers is lower than that of the natural

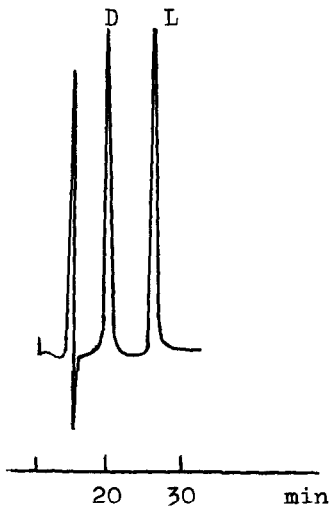

Fig. 2. Separation of enantiomers of α-CF_3-alanine. Column, Si 100 Polyol Chiral Pro-Cu, 5 μm (250 × 4.6 mm I.D.); mobile phase, $5 \cdot 10^{-3}$ *M* $CuSO_4$; flow-rate, 1 ml/min; temperature, ambient; wavelength, 225 nm.

compounds. In most instances the selectivity of separation on the hydroxyproline column is higher than that on the proline column. For example, whereas on the proline column there is no separation of Leu, Nle, Nva or Ala, the separation is excellent on the hydroxyproline column. However, the separation of α-CF_3Ala and α-CF_3Asp enantiomers is not possible on the hydroxyproline column, whereas it is easily achieved on the proline column. In addition, the separation of α-CF_3Asp, Phe and α-CF_3Phe is

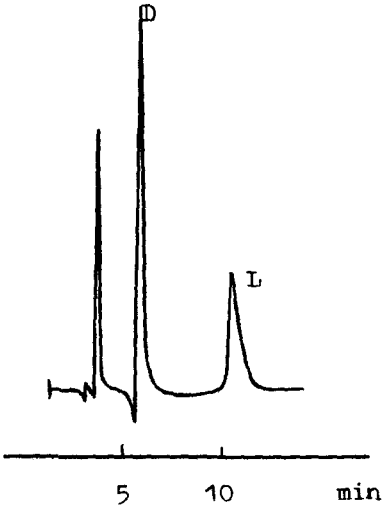

Fig. 3. Separation of enantiomers of α-CF_3-phenylglycine. Column, Nucleosil Chiral-1, 5 μm (250 × 4 mm I.D.); mobile phase, A–B (75:25), where A = $5 \cdot 10^{-3}$ *M* $CuSO_4$–10% (v/v) ethanol and B = $5 \cdot 10^{-3}$ *M* $CuSO_4$ (pH 3.0, HCl); flow-rate, 1 ml/min; temperature, 50°C.

TABLE I

SELECTIVITY PARAMETERS FOR THE SEPARATION OF L-AMINO ACIDS

Compound	$\Delta(\Delta G)^a$ *(kJ/mol)*	
	Proline column	*Hydroxyproline column*
α-CF_3Phe–Phe	1.26	0.84
α-CF_3Nle–Nle	1.66	1.81
α-CF_3Ala–Ala	1.63	2.88
α-CF_3Nva–Nva	2.46	2.99
α-CF_3Leu–Leu	2.24	2.72
α-CF_3Asp–Asp	2.04	4.43

[a] $\Delta(\Delta G) = RT \ln \alpha$.

more selective on the proline column. Replacing the benzene ring in Phe for the hydrophilic heterocycle of uracil [villardine(uracilylalanine) natural alkaloid[5]] leads to a considerable decrease in the retention and selectivity of separation of enantiomers on the proline column and to a complete loss of selectivity on the hydroxyproline column. Enantioselectivity may by caused by coordination and hydrophobic interactions and also hydrogen bonds between ligands[6]. As in our case ethanol added to the mobile phase (0.5–50%, v/v) has no effect on the selectivity of enantiomer separation, we can assume that hydrophobic interactions have no appreciable effect on this process.

The results obtained enable optimum conditions and a sorbent for the complete separation of amino acid enantiomers to be chosen.

TABLE II

SEPARATION OF AMINO ACID ENANTIOMERS ON PROLINE AND HYDROXYPROLINE SORBENTS

Mobile phase, $5 \cdot 10^{-3}$ *M* $CuSO_4$; temperature, 20°C.

Compound	*Proline column*		*Hydroxyproline column*	
	k'_L	α^a	k'_L	α^a
Phe	16.8	2.0	3.7	1.8
Leu	5.8	1.0	1.4	2.1
Ala	4.3	1.0	0.5	1.7
Nle	7.3	1.0	3.8	1.9
Nva	4.2	1.0	1.6	2.4
Asp	2.5	1.0	0.5	1.0
α-CF_3Phe	28.0	6.0	5.2	1.3
α-Trifluoroaminobutyric acid	7.6	1.5	3.2	1.6
α-CF_3Nle	14.3	1.5	7.9	1.9
3,3,3-Trifluoroalanine	5.1	1.3	1.2	2.4
α-CF_3Nva	11.7	1.4	4.8	1.8
α-CF_3Ala	8.3	1.8	1.6	1.0
α-CF_3Leu	14.3	1.6	4.7	2.0
α-CF_3Asp	5.7	1.6	3.0	1.2
Villardine	6.2	1.2	0.5	1.0

[a] $\alpha = k'_L k'_D$.

REFERENCES

1 C. T. Walsh, *Annu. Rev. Biochem.*, 53 (1984) 493.
2 J. R. Guerson and M. J. Adam, *J. Chromatogr.*, 325 (1985) 103.
3 I. W. Keller and B. I. Hamilton, *Tetrahedron Lett.*, 27 (1986) 1249.
4 V. A. Soloshonok, I. I. Herus, Yu. L. Yagupolskii and V. P. Kukhar, *Zh. Org. Khim.*, 23 (1987) 1441.
5 Yu. I. Shwatchkin and M. P. Azarova, *Zh. Obshch. Khim.*, 34 (1964) 407.
6 P. Roumeliotus, K. Unger, A. Kurganov and V. Davankov, *J. Chromatogr.*, 225 (1983) 51.