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Note

Ligand-exchange chromatography of amino acid racemates on polystyrene sorbents containing L-methionine-*d*-sulphoxide or L-methionine-*l*-sulphoxide groups

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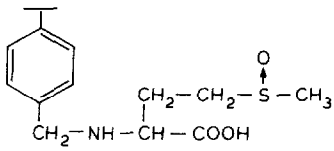
In the past few years ligand-exchange chromatography (LEC) has attracted increasing interest as a promising method for resolution of racemates. Matrixes based on polymers of styrene[1], acrylamide[2], methacrylic acid[3], as well as silica gel[4,5] were used for binding chiral chelating ligands, primarily amines, amino acids and their derivatives. On the basis of experimental data it has been shown that the enantioselective formation of sorption complexes, which determine the resolving ability of sorbents, is influenced by several factors and especially by fixed ligand structure. When L-hydroxyproline[6] and L-allohydroxyproline[7], having opposite configurations at the γ-carbon atoms, were used as resin-bound ligands, the results of enantiomeric resolutions differed significantly. In this case the difference arose from the additional coordination of the γ-hydroxy group of allohydroxyproline in the axial position of the chelated copper(II) ion. However, it is difficult to predict the rôle of the configuration of atoms which are not involved in chelation of the central metal ion. To examine this point, we synthesized sorbents containing L-methionine-*d*-sulphoxide (I) and L-methionine-*l*-sulphoxide (II) groups in a macronet polystyrene matrix. The sulphoxide group in methionine sulphoxide is supposed not to participate in the coordination of nickel ions[8].



The polymers were charged with copper or nickel for LEC of amino acids.

EXPERIMENTAL

Synthesis

L-Methionine-*d,l*-sulphoxide was prepared as described[9] by oxidation of L-methionine (optical purity 96%) with hydrogen peroxide in acetic acid. The oxidation proceeds with some enantiospecificity, and the specific rotation of the product was $[\alpha]_D^{20} = +75.4°$ (c = 2%, 1 M HCl), corresponding to a 72% content of the L-*d*

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diastereoisomer. The diastereoisomeric sulphoxides were separated by crystallization of picrates[10]. The resulting L-*d* diastereoisomer possessed a specific rotation of $[\alpha]_D^{20} = +113.4°$ (c = 2%, 1 *M* HCl), corresponding to a purity of 94%. Ethyl ester hydrochlorides of these diastereoisomers were prepared by adding the amino acids to 2 *M* HCl solution in ethanol and allowing the mixture to stand for 24 h at 25°C. The specific rotation of the ethyl ester hydrochloride of the L-*d* diastereoisomer was $[\alpha]_D^{20} = +64.8°$ (c = 4%, water), m.p. 166–168°C; that of the L-*l* diastereoisomer was $[\alpha]_D^{20} = +21.3°$ (c = 4%, water), m.p. 158–162°C.

The polymeric sorbents were prepared by treating chloromethylated styrene copolymer (11 mol % methylene cross-linking, chlorine content 21%, 1 mol of chloromethyl groups) with the ethyl ester hydrochlorides of L-*d* and L-*l* sulphoxides (2 mol) in the presence of NaI (0.3 mol) and $NaHCO_3$ (8 mol) in dioxane–methanol (6:1 v/v) at 60°C. The reaction time was 16 h. The sulphur and nitrogen contents of the reaction products were 6.6 and 3.1% for the L-*d* diastereoisomer and 7.0 and 3.2% for the L-*l* diastereoisomer, respectively. The ester groups were hydrolysed with 1 *M* KOH solution in dioxane–water (1:1 v/v) at 20°C for 20 h. After hydrolysis the contents of sulphur and nitrogen amounted to 6.5% (2.0 mmol/g) and 3.1% (2.3 mmol/g) for the L-*d* sulphoxide and 6.7% (2.1 mmol/g) and 3.2% (2.3 mmol/g) for the L-*l* sulphoxide, respectively. The polymers were loaded with copper(II) ions using a 0.1 *M* solution of copper(II) chloride in 0.5 *M* aqueous ammonia and with nickel(II) ions using a 0.05 *M* solution of nickel chloride in 0.5 *M* aqueous ammonia. The copper contents were 0.74 and 0.77 mmol/g (stationary ligand: metal ion ratios 2.7:1 and 3.0:1) for sorbents I and II, respectively. The nickel contents were 0.81 and 0.77 mmol/g (stationary ligand: metal ion ratios 2.5:1 and 2.7:1) for sorbents I and II, respectively.

Chromatography

Stainless-steel columns (20 × 0.4 cm I.D.), containing ≈2 g of dry sorbent (particle size < 50 μm, swelling capacity in water ≈100%), and a Uvicord-III detector (λ = 206 nm) were used for chromatography. The eluents were 1.0 *M* and 0.1 *M* aqueous ammonia solutions (containing $5 \cdot 10^{-5}$ *M* and $2 \cdot 10^{-5}$ *M* copper ions respectively), 0.1 *M* ammonia carbonate solution (pH 9.5) (containing $2 \cdot 10^{-6}$ *M* nickel ions) and water. The flow-rate was 7 ml/h. The amount of amino acid introduced was 0.4 μmol. The k'_L and k'_D values obtained for the individual enantiomers of proline and resolved racemic proline were identical.

RESULTS AND DISCUSSION

The results of the ligand-exchange chromatography on the copper(II)-loaded polymers are summarized in Table I. Comparing α values ($\alpha = k'_D/k'_L$) for the same amino acids, it is seen that the enantioselectivity is in most cases somewhat higher for polymer II containing L-*l* sulphoxide groups (except for Leu, Tyr, Phe). However, the differences observed in α are mostly insignificant, the largest amounting to 0.27 (accuracy of determination ±0.05). On the basis of these experimental data we can conclude that the configuration at the sulphur atom in methionine sulphoxide has little influence on enantioselectivity in its mixed ligand complexes formed by copper(II) ions with amino acids. As copper(II) ion forms square planar com-

TABLE I

CHROMATOGRAPHY OF AMINO ACID ENANTIOMERS ON THE COPPER(II) FORM OF SORBENTS I AND II, CONTAINING L-*d* AND L-*l* METHIONINE SULPHOXIDE GROUPS

Eluents: aqueous ammonia solutions (mol/l) and water. Superscript −1 indicates reversed elution order of enantiomers.

Amino acid	*Sorbent I*				*Sorbent II*			
	k'_D	k'_L	$\alpha = k'_D/k'_L$	*Eluent*	k'_D	k'_L	$\alpha = k'_D/k'_L$	*Eluent*
Proline	5.66	4.63	1.22	0.3	13.0	7.83	1.66	0.1
	10.8	8.00	1.35	0.2	21.4	12.0	1.78	0.05
	15.6	10.2	1.53	0.1				
	24.4	14.2	1.72	0.05				
Alanine	2.92	2.73	1.07	0.1	2.73	2.29	1.19	0.1
Valine	9.09	7.27	1.25	0.1	7.22	5.60	1.29	0.1
Leucine	25.1	19.3	1.30	0.1	24.2	20.0	1.21	0.1
Methionine	12.7	11.6	1.09	0.1	18.3	14.3	1.28	0.1
Threonine	1.02	0.82	1.24	0.1	0.85	0.67	1.27	0.1
Tyrosine	3.64	2.54	1.43	0.1	5.36	4.40	1.22	0.1
Phenylalanine	40.0	27.1	1.48	0.1	37.6	26.8	1.40	0.1
Histidine	10.0	15.5	1.55^{-1}	1.0	11.0	20.0	1.82^{-1}	1.0
Aspartic acid	7.00	5.50	1.27	Water	7.50	6.00	1.25	Water

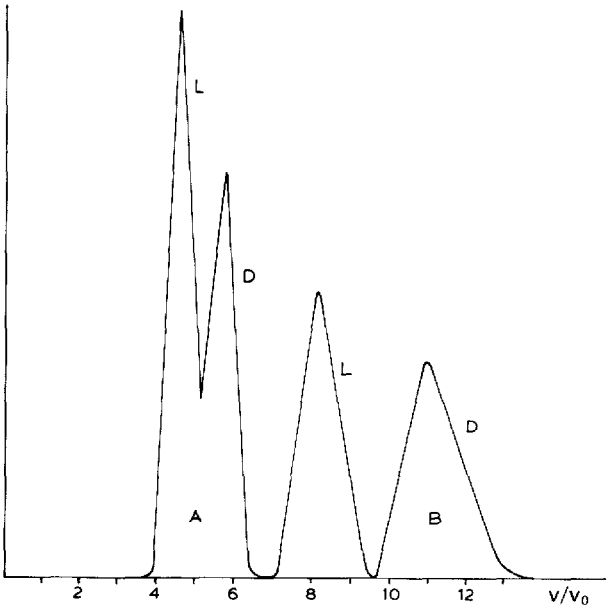

Fig. 1. Chromatography of racemic proline on the copper(II) form of sorbent I. Eluents: A, 0.3 *M* aqueous ammonia solution; B, 0.2 *M* aqueous ammonia solution.

TABLE II

CHROMATOGRAPHY OF AMINO ACID ENANTIOMERS ON THE NICKEL(II) FORM OF SORBENTS I AND II, CONTAINING L-*d* AND L-*l* METHIONINE SULPHOXIDE GROUPS

Eluents: 0.1 *M* $(NH_4)_2CO_3$(pH 9.5) (A) or water. Superscript -1 indicates reversed elution order of enantiomers.

Amino acid	*Sorbent I*				*Sorbent II*			
	k'_D	k'_L	α	*Eluent*	k'_D	k'_L	α	*Eluent*
Proline	1.57	1.14	1.38	A	2.33	2.17	1.07	A
Alanine	1.12	1.12	1.00	A	1.71	1.71	1.00	A
Valine	2.14	2.14	1.00	A	2.95	2.86	1.03	A
Leucine	5.53	5.00	1.11	A	6.86	6.40	1.07	A
Threonine	2.67	2.33	1.14	A	4.57	3.81	1.20	A
Tyrosine	27.3	22.7	1.20	A	28.7	24.0	1.20	A
Phenylalanine	30.0	24.7	1.21	A	57.6	48.4	1.19	A
Histidine	18.0	40.0	2.22^{-1}	A	35.0	43.0	1.23^{-1}	A
Aspartic acid	0.35	0.43	1.23^{-1}	A	0.47	0.50	1.06^{-1}	A
	2.95	4.54	1.54^{-1}	Water	5.80	7.35	1.28^{-1}	Water

plexes, the presence of the sulphoxide group in the amino acid side chain has no influence on the structure.

D,L-Proline was eluted using ammonia solutions of different concentrations. On reducing the concentration of ammonia both the capacity factors, k', and α values were found to increase from $\alpha = 1.22$ (0.3 *M* NH_3) up to 1.72 (0.05 *M* NH_3) and from $\alpha = 1.66$ (0.1 *M* NH_3) up to 1.78 (0.05 *M* NH_3) on polymers I and II, respectively (Fig. 1).

The results of the LEC on the nickel(II) form of the sorbents are given in Table II. While coordination of the sulphoxide group to nickel ion is considered to be unlikely, because this would result in the formation of unstable seven membered chelate rings, the chromatographic results for tridentate His and Asp still pointed to a significant influence of the sulphur atom configuration: α values for His were 0.45 and 0.81 and those for Asp were 0.65 and 0.79 on polymers I and II, respectively. Of the other amino acids, the α values for Pro were 1.38 and 1.07 on polymers I and II, respectively. For the remainder, the differences in α were small and, as a rule, the enantioselectivity was higher for polymer I (except for Thr).

As a rule, D-amino acids were more strongly retained than L-enantiomers, but His (copper and nickel forms of the sorbents) and Asp (nickel form of the sorbents) showed the opposite elution order of the isomers, *i.e.*, sorption complexes with two ligands of the same L-configuration appeared to be more stable.

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