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EFFECT OF THE HYDROPHOBIC SPACER IN BONDED [Cu(L-HYDROXYPROLYL)ALKYL]$^+$ SILICAS ON RETENTION AND ENANTIOSELECTIVITY OF α-AMINO ACIDS IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

The following chiral ligands were bonded to silica: [Cu(L-hydroxyprolyl)methyl)$^+$ on LiChrosorb Si 60 (1), [Cu(L-hydroxyprolyl)methyl]$^+$ on LiChrosorb Si 100 (2) and [Cu(L-hydroxyprolyl)*n*-octyl]$^+$ on LiChrosorb Si 100 (3). The packings contained residual iodomethyl- and ω-iodooctyl groups at the surface. Studies on packing 1 and 2 under comparable conditions in eluents containing 10^{-4} *M* copper acetate showed a higher retention on 1 than on 2 but a much better enantioselectivity in the latter case. The retention of enantiomers on all packings examined was found to be governed by the eluent pH and methanol content as well as by the concentration of ammonium acetate. The variation of the hydrophobic spacer from methyl, to propyl and *n*-octyl in the bonded bis(amino acidato)copper complex permitted a sensitive control of retention and gave rise to unique changes in the enantioselectivities which are assumed to be attributable to multiple solute–surface interactions, *i.e.*, complexation, ion-exchange and hydrophobic interactions.

INTRODUCTION

Column chromatography has proved to be a promising technique for the separation of enantiomers in many fields[1–3]. Interest has focused on the synthesis of chemically modified silicas carrying a bonded optically active amino acid fragment as chiral ligand[4–13]. In earlier studies in our laboratories we examined[14] a bonded silica composed of L-proline and 4-L-hydroxyproline linked via the amino group to an *n*-propyl spacer anchored to the surface, and employing Cu^{2+} as complexing metal ion. The retention behaviour of α-amino acid enantiomers on these packings was rationalized in terms of complexation to a *trans*-bis(amino acidato)copper complex and additional interactions via complexation of free ligand sites, hydrophobic interaction between the radical R to the α-amino acid and the *n*-propyl spacer of the bonded ligand, hydrogen bonding, ionic interactions, etc.

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As an extension of these studies, the systematic variation of the type of hydrophobic spacer X in the [Cu(L-hydroxyprolyl)X]$^+$ complex bonded at the silica surface was undertaken[15].

The present paper examines the influence of the chain length of the *n*-alkyl spacer in the L-hydroxyprolyl chiral ligand bonded to a 6- and 10-nm pore size silica on the retention of α-amino acid enantiomers with different eluent compositions.

EXPERIMENTAL

Preparation of packings

Packing 1. Ten grams of LiChrosorb Si 60, d_p = 10 μm (E. Merck, Darmstadt, G.F.R.), were heated for 24 h in a refluxing solution of 8 g of chloromethyltrimethoxysilane (kindly supplied by Degussa, Hanau, G.F.R.) in 300 ml of *n*-octane. After removal of the excess of silane, and washing, a solution of 5 g of L-hydroxyproline methyl ester and 0.5 g NaI in dioxane–methanol (6:1) was added. Heating under reflux was continued for 12 h. After washing, the packing was treated with a copper acetate solution at 323°K to hydrolyze the ester groups. Elemental analysis gave 2.25% C, 0.75% H and 0.49 % N.

Packing 2. Reaction was carried out on LiChrosorb Si 100 (E. Merck) under exactly the same conditions as stated for packing 1. Elemental analysis: 2.27 % C, 0.63 % H and 0.28 % N.

Packing 3. LiChrosorb Si 100, d_p = 10 μm (E. Merck), was treated with ω-bromooctyltrichlorosilane (Petrarch, PA, U.S.A.) following the procedure described above. Elemental analysis: 13.06 % C, 2.18 % H and 1.69 % N.

Chromatographic measurements.

Columns were packed by the high-viscosity slurry technique with *n*-heptane as second liquid. After washing with 2-propanol the columns were loaded with Cu^{2+} by flushing the column with concentrated aqueous copper acetate (reagent grade, E. Merck). The loading procedure was always repeated when the eluent composition was changed.

The α-amino acids (Sigma, Munich, G.F.R. and Degussa) were aspartic acid (Asp), glutamic acid (Glu), histidine (His), alanine (Ala), asparagine (Asn), glutamine (Gln), serine (Ser), proline (Pro), citruline (Cit), threonine (Thr), valine (Val), 3,4-dihydroxyphenylalanine (DOPA), lysine (Lys), norvaline (Nval), tyrosine (Tyr), methionine (Met), arginine (Arg), isoleucine (Ile), leucine (Leu), norleucine (Nleu), ethionine (Eth), phenylalanine (Phe) and tryptophan (Trp).

The eluent was 0.0001 *M* copper acetate in all cases. The pH was varied between 4 and 6 by adding acetic acid–ammonium acetate; the ammonium acetate concentration used was 0.001, 0.01 or 0.1 *M*. Methanol, acetonitrile and tetrahydrofuran were employed as organic solvents at a constant composition of 50:50 (v/v) in aqueous solution. The methanol content was also increased stepwise, *viz.*, 10:90, 30:70, 50:50 and 75:25 (v/v). The liquid chromatograph was a Hewlett-Packard Model 1084 A fitted with a UV photometer operating at a fixed wavelength of 254 nm. The column temperature was set at 298, 323 or 348°K. The enantioselectivity, α, was expressed by the ratio k'_D/k'_L, where k'_D is the capacity factor of the D-enantiomer and k'_L that of the L-

enantiomer. The dead time t_0 was measured by applying a slightly modified mobile phase.

RESULTS AND DISCUSSION

Properties of chiral bonded phases

Synthetic routes to these types of chiral packings consist of either (i) preparing the optically active silane by treament of an appropriate haloalkylsilane with the methyl ester of L-hydroxyproline and subsequently binding this to the silica, or (ii) binding the haloalkylsilane to the silica, followed by reaction of the modified silica with the methyl ester of L-hydroxyproline in the presence of sodium iodide. The various aspects of the manufacture of chiral bonded phases and their consequent surface structures will be discussed elsewhere[16]. In the present investigation the second route (ii) was chosen, yielding the surface structure illustrated in Fig. 1 for packings 1 and 3. Three surface functional groups can be distinguished: (L-hydroxyprolyl)alkyl groups capable of complexation with Cu^{2+}, unreacted iodoalkyl groups and unreacted silanol groups. Problems arise in the estimation of the relative proportions of these groups: the carbon content contains contributions from both the iodo- and (L-hydroxyprolyl)alkyl groups; the nitrogen and iodine contents are too small to discriminate reliably between the groups. Even when the (L-hydroxyprolyl)alkyl groups were complexed with Cu^{2+}, the capacity for Cu^{2+} does not remain constant but changes according to the pH of the eluent, etc. Thus an intensive examination is required in order to establish any accurate model for such a complex surface.

On increasing the *n*-alkyl chain length of the spacer from methyl to propyl and *n*-octyl, the hydrophobic character of the surface successively increases. At the same time the terminating chiral groups extend further into the pore space and become more flexible in the solvated state. The change in hydrophobicity and steric orientation of

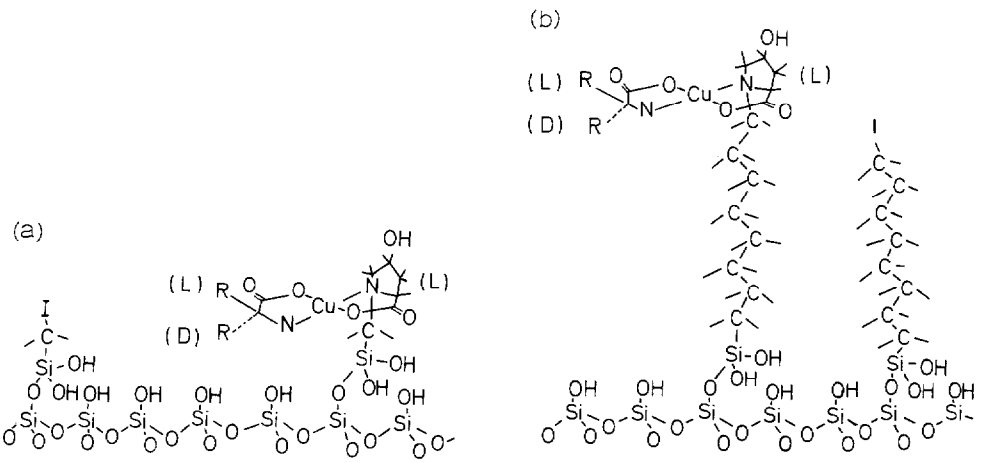

Fig. 1. Tentative models of the surface of (L-hydroxylpropyl) methyl (a) and ω-(L-hydroxyprolyl)*n*-octyl (b) silica in their complexed forms, illustrating the various types of surface functional groups.

TABLE I

EFFECT OF ELUENT pH ON THE RETENTION AND ENANTIOSELECTIVITY OF α-AMINO ACID ENANTIOMERS

Columns: 100 × 4.6 mm. Packings: 1–3, d_p = 10 μm. Eluent: 10^{-4} *M* copper acetate + 10^{-2} *M* ammonium acetate; pH adjusted with acetic acid. Column temperature: 323°K.

Amino acid	*[Cu(L-hydroxyprolyl)methyl]⁺ silica (Si 60) pH of eluent*									*[Cu(L-hydroxyprolyl)methyl]⁺ silica (Si 100) pH of eluent*									*[Cu(L-hydroxyprolyl)n-octyl]⁺ silica (Si 100) pH of eluent*								
	4.5			*5.0*			*5.5*			*5.0*			*5.5*			*6.0*			*4.7*			*5.0*			*5.4*		
	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α
Asp	0.44	0.44	1.00	1.10	1.03	0.94	0.76	0.71	0.93	0.13	0.14	1.08	0.05	0.05	1.00	0.20	0.20	1.00	1.48	1.55	1.05	2.01	2.13	1.06	2.92	3.18	1.09
Glu	0.39	0.42	1.09	1.02	1.13	1.11	0.77	0.82	1.06	0.10	0.25	2.50	0.05	0.13	2.60	0.20	0.20	1.00	1.02	1.02	1.00	1.57	1.57	1.00	2.78	2.78	1.00
His	1.62	1.08	0.67	3.80	2.33	0.61	4.62	3.08	0.67	1.70	1.27	0.75	1.97	1.78	0.90	3.10	2.87	0.92	1.46	1.22	0.83	2.22	1.92	0.86	3.75	1.82	0.48
Ala	0.47	0.51	1.08	1.40	1.47	1.05	1.79	1.86	1.04	0.48	0.58	1.21	0.50	0.58	1.16	1.13	1.13	1.00	0.17	0.24	1.42	0.40	0.58	1.45	0.85	1.12	1.32
Asn	0.81	0.77	0.95	2.05	1.76	0.86	1.71	1.71	1.00	0.52	0.80	1.54	0.41	0.57	1.39	1.08	1.15	1.06	0.55	0.50	0.91	0.89	0.82	0.92	1.83	1.68	0.92
Ser	0.57	0.55	0.96	1.72	1.46	0.85	1.66	1.54	0.93	0.65	0.65	1.00	0.45	0.45	1.00	1.08	1.08	1.00	0.46	0.42	0.91	0.82	0.74	0.90	1.92	1.78	0.93
Pro	0.79	0.90	1.14	1.93	2.51	1.30	3.42	4.64	1.36	0.63	0.90	1.43	1.05	1.47	1.40	2.28	2.35	1.03	0.56	1.10	1.96	1.07	2.06	1.93	2.85	5.25	1.84
Thr	0.79	0.85	1.07	2.14	1.94	0.91	2.69	2.48	0.92	0.73	1.00	1.37	0.57	0.65	1.14	1.22	1.30	1.06	0.71	0.71	1.00	1.14	1.16	1.02	2.18	2.17	1.00
Val	0.80	0.80	1.00	2.10	2.12	1.01	3.30	3.47	1.05	0.80	0.90	1.12	1.42	1.18	0.83	2.65	1.58	0.60	1.61	1.53	0.95	2.81	2.71	0.96	5.98	5.83	0.97
Lys	0.99	0.92	0.93	2.77	2.61	0.94	6.57	6.23	0.95	1.42	1.48	1.04	2.82	2.72	0.96	—	—	—	-0.09	-0.12	1.33	0.07	0.02	0.29	0.42	0.32	0.75
Tyr	0.75	0.62	0.83	2.30	1.52	0.66	2.42	1.59	0.66	1.37	0.95	0.69	1.01	0.80	0.79	1.55	1.33	0.86	7.39	5.94	0.80	12.25	9.72	0.79	24.3	19.2	0.76
Met	0.91	0.97	1.07	2.87	2.82	0.98	3.32	3.38	1.02	1.23	1.40	1.14	1.53	1.53	1.00	2.21	2.56	1.16	2.75	2.71	0.98	4.05	4.56	1.13	8.91	8.72	0.98
Arg	1.21	1.21	1.00	3.40	3.44	1.01	7.90	8.00	1.02	2.05	1.78	0.87	3.53	3.37	0.95	—	—	—	0.05	0.09	1.80	0.31	0.39	1.26	0.97	1.28	1.32
Ile	0.88	0.85	0.97	2.45	2.51	1.02	3.67	3.87	1.05	1.23	1.23	1.00	—	—	—	3.15	3.15	1.00	3.15	3.15	1.00	5.86	5.94	1.01	12.13	12.27	1.01
Leu	0.97	0.89	0.92	2.83	3.02	1.07	3.77	3.75	0.99	1.30	1.03	0.79	1.67	1.45	0.87	2.60	2.43	0.94	2.78	2.96	1.06	4.80	5.14	1.07	10.2	10.9	1.07
Phe	1.10	1.02	0.93	3.35	2.87	0.86	3.43	3.28	0.96	1.32	1.32	1.00	1.58	1.78	1.13	2.00	2.35	1.17	10.43	9.42	0.90	17.60	15.45	0.88	38.35	31.02	0.81
Trp	1.58	1.10	0.70	4.69	2.69	0.57	4.33	3.32	0.77	2.42	1.58	0.65	—	—	—	2.22	1.95	0.88	21.56	14.19	0.66	—	—	—	—	—	—

the bonded moiety, relative to other vicinal surface groups, may be expected to give rise to a unique enantioselectivity of the phase systems.

Parameters controlling retention and enantioselectivity of α-amino acid enantiomers

Type of supporting silica. Both LiChrosorb Si 60 and Si 100 (with surface areas of 501 and 316 m^2/g respectively, according to the three-parameter BET equation[17]) are representative packings widely used for surface modification, and hence were chosen for this examination. L-Hydroxyprolylmethyl groups were preferred for bonding to these silicas because the resulting spacer (methylene) is short compared to propyl and *n*-octadecyl; long *n*-alkyl groups are known to cause a drastic reduction of specific surface area and specific pore volume and a diminution of the mean pore diameter of the initial silica on modification[18]. Considering packings 1 and 2, which carry the same types of functional groups, a significant difference is seen in that the surface energy is higher on packing 1 than on packing 2.

The retention data collected in Tables I–IV indicate that in all cases retention of enantiomers is greater on packing 1 than on packing 2. This is also valid on variation of pH, methanol content and type of organic solvent, under otherwise constant conditions. However, inspection of the enantioselectivity shows higher α values for packing 2 than for packing 1. In addition, for most of the α-amino acid enantiomers the elution sequence remained unchanged. Exceptions are: Asn, Thr and Phe (in Table I) at pH 5.0, 5.5 and 6.0 (D < L on packing 1, L < D on packing 2); Phe, Ile, Arg, Lys and Asn (in Table II) with 0:100, 10:90, 30:70 and 50:50 (v/v) methanol–water; Phe, Ile, Arg, Lys, Asn and Asp (in Table III) with methanol–water, acetonitrile–water and tetrahydrofuran–water (all 50:50, v/v). The observation that selectivity changes occured for all types of α-amino acids (basic, acidic, hydrophobic) justifies the assumption that several effects operating in combination bring about the steric recognition of enantiomers on the phase system examined. The results clearly demonstrate the dependence of retention and enantioselectivity on the type of silica employed for the bonding procedure.

The pH of the eluent (see Table I). It was demonstrated in earlier studies on 3-(L-hydroxyprolyl)propyl silica[14] that a pH range of 4–6 in the eluent was the optium for retention of α-amino acid enantiomers, with respect to peak shape and column efficiency. On inspection of the retention on (L-hydroxyprolyl)methyl- and (L-hydroxyprolyl)*n*-octyl-silica at pH 5.0 and incorporating the data on packing III from ref. 14, it follows that $k'_{\text{methyl silica}} < k'_{\text{propyl silica}} < k'_{n\text{-octyl silica}}$ for the majority of enantiomeric solutes. Assuming that complexation interactions remain the same on all three packings for a given enantiomer, the enhancement of k' is due to increasing hydrophobic interactions, these being proportional to the *n*-alkyl chain length of the spacer. The typical changes of solute k' on varying the eluent pH obtained on (L-hydroxyprolyl)propyl silica in our previous study[14] were also observed here on methyl and *n*-octyl silicas.

On (L-hydroxyprolyl)methyl silica the largest retention occurred for the basic α-amino acids, His, Lys and Arg, and for the hydrophobic acids, Phe, Trp, Leu and Ile. Met, Val, Pro and Ala were moderately retarded and the acidic α-amino acids gave the smallest retention. This retention order reflects the varied nature of the interactions involved (*viz.* ion exchange, exchange, complexation, hydrophobic, etc.) and their

TABLE II

EFFECT OF ELUENT METHANOL CONTENT ON RETENTION AND ENANTIOSELECTIVITY OF α-AMINO ACID ENANTIOMERS

Conditions as in Table I, pH 5.0.

Amino acid	*[Cu(L-hydroxyprolyl)methyl]⁺ silica (Si 60) methanol–aqueous eluent (v/v)*												*[Cu(L-hydroxyprolyl)methyl]⁺ methanol–aqueous eluent (v/v)*					
	0:100			*10:90*			*30:70*			*50:50*			*0:100*			*10:90*		
	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α
Asp	0.44	0.44	1.00	1.00	1.00	1.00	1.13	1.13	1.00	2.64	2.52	0.95	0.13	0.14	1.08	0.10	0.10	1.00
Glu	0.39	0.42	1.08	0.88	0.96	1.09	0.74	0.84	1.14	2.04	2.33	1.14	0.10	0.25	2.50	0.10	0.10	1.00
His	1.62	1.08	0.67	3.42	1.85	0.54	3.58	1.71	0.48	4.42	2.02	0.46	1.70	1.27	0.75	1.26	0.95	0.75
Ala	0.47	0.51	1.08	1.18	1.26	1.07	0.79	0.87	1.10	2.10	2.34	1.11	0.48	0.58	1.21	0.32	0.42	1.31
Asn	0.81	0.77	0.96	1.94	1.80	0.93	1.74	1.62	0.93	4.03	3.40	0.84	0.52	0.80	1.54	0.42	0.60	1.43
Ser	0.57	0.55	0.96	1.52	1.41	0.93	1.17	1.11	0.95	3.00	2.65	0.88	0.65	0.65	1.00	0.45	0.45	1.00
Pro	0.79	0.90	1.14	1.46	2.07	1.42	0.97	1.22	1.26	2.33	3.92	1.68	0.63	0.90	1.43	0.42	0.62	1.47
Thr	0.79	0.85	1.07	1.91	1.97	1.03	1.53	1.68	1.10	3.41	3.51	1.03	0.73	1.00	1.37	0.57	0.75	1.32
Val	0.80	0.80	1.00	1.66	1.68	1.00	1.04	1.05	1.01	2.49	2.52	1.01	0.80	0.90	1.12	0.52	0.48	0.92
Lys	0.99	0.92	0.93	2.59	2.35	0.91	1.47	1.32	0.90	4.90	4.15	0.85	1.42	1.48	1.04	1.08	1.13	1.05
Tyr	0.75	0.62	0.82	1.88	1.44	0.77	1.30	1.06	0.81	3.13	2.43	0.78	1.37	0.95	0.69	0.92	0.73	0.80
Met	0.91	0.97	1.07	1.87	2.03	1.09	1.18	1.39	1.18	2.66	3.00	1.13	1.23	1.40	1.14	0.77	0.77	1.00
Arg	1.21	1.21	1.00	2.97	3.02	1.02	1.54	1.58	1.02	4.48	4.64	1.03	2.05	1.78	0.87	1.35	1.35	1.00
Ile	0.88	0.85	0.96	1.66	1.63	0.98	0.98	0.94	0.96	2.20	2.13	0.97	1.23	1.23	1.00	0.67	0.70	1.05
Leu	0.97	0.89	0.92	1.89	1.74	0.92	1.12	1.01	0.90	2.56	2.30	0.90	1.30	1.03	0.79	0.70	0.57	0.81
Phe	1.10	1.02	0.93	2.30	1.97	0.86	1.41	1.26	0.89	3.04	2. 5	0.87	1.32	1.32	1.00	0.77	0.70	0.91
Trp	1.58	1.10	0.70	3.77	2.40	0.64	2.42	1.57	0.65	5.27	3.47	0.66	2.42	1.58	0.65	1.33	0.97	0.73

individual contributions to the net interaction. Basic α-amino acids involved in an ion-exchange process with weakly acidic surface silanols were seen to be progressively retained on raising the pH of the eluent. This is in accord with the increasing deprotonation of silanols to siloxanyl ions in this pH range. Acidic α-amino acids are not able to undergo ion exchange and hence show a much smaller increase in retention. The comparison of capacity factors of Phe and Ala, which have the same pK values for their functional groups, indicates the order of hydrophobic interactions.

On varying the pH on (L-hydroxyprolyl)*n*-octyl silica, the same retention behaviour was observed as for the methyl silica. However, due to the longer *n*-alkyl chain, hydrophobic α-amino acids such as Tyr, Phe, Leu and Trp exhibited the greatest retention of all enantiomers. A drastic change occurs for the basic α-amino acids: for example, Lys was eluted with the column dead volume from packing 3. This may be explained by the possibility that the (L-hydroxyprolyl)*n*-octyl chains cover the surface with such a dense layer that residual silanols become inaccessible and for some unknown reason complexation does not occur to a noticeable extent.

Both packings 2 and 3 offer an appreciable enantioselectivity. The elution sequence on both packings was the same, *i.e.*, L < D or D < L. The only exceptions were Asn, Val, Arg, Leu and Phe. Comparison of the elution order on packing 2 with that on (L-hydroxyprolyl)propyl silica from ref. 14 gave differences only with Asp, Arg, Leu and Phe. The same was true for α values on (L-hydroxyprolyl)propyl silica and packing 3; exceptions to the elution order were found for Asp and Val.

Methanol content of the eluent (Table II). The variation of the methanol content of the eluent provides a simple means of estimating the extent of hydrophobic inter-

silica (Si 100)						*[Cu(L-hydroxyprolyl) n-octyl]$^+$ silica (Si 100) methanol–aqueous eluent (v/v)*														
30:70			*50:50*			*0:100*			*10:90*			*30:70*			*50:50*			*75:25*		
k'_L	k'_D	α	k'_L	k'_L	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α
0.25	0.30	1.20	0.55	0.62	1.13	2.01	2.13	1.06	3.05	3.27	1.07	6.32	6.45	1.02	10.70	10.58	0.99	21.82	21.22	0.97
0.20	0.52	2.60	0.58	0.82	1.41	1.57	1.57	1.00	2.43	2.30	0.95	4.62	4.65	1.01	6.42	6.47	1.01	8.72	8.34	0.96
1.60	1.07	0.67	1.70	1.13	0.67	2.22	1.92	0.86	2.35	3.10	1.32	2.43	5.72	2.30	2.77	7.85	2.83	5.60	10.82	1.93
0.53	0.63	1.19	0.80	1.00	1.25	0.40	0.58	1.45	0.73	0.83	1.14	.	.	.	2.00	1.68	0.84	2.94	3.50	1.19
0.60	1.05	1.75	0.93	1.33	1.43	0.89	0.82	0.92	1.12	1.05	0.94	1.55	1.38	0.89	2.12	1.82	0.86	4.76	3.94	0.83
0.77	0.67	0.87	0.97	1.00	1.03	0.82	0.74	0.90	1.23	1.07	0.87	1.90	1.60	0.84	2.55	2.38	0.93	5.44	4.96	0.91
0.47	0.75	1.60	0.85	1.78	2.09	1.07	2.06	1.92	1.35	2.68	1.99	1.82	3.68	2.02	1.97	4.18	2.12	3.20	6.80	2.12
0.72	1.08	1.50	1.18	1.62	1.37	1.14	1.16	1.02	1.37	1.42	1.04	1.72	1.82	1.06	2.92	2.62	0.90	5.42	4.96	0.91
0.75	0.93	1.24	1.05	1.00	0.95	2.81	2.71	0.96	3.05	2.93	0.96	3.31	3.27	0.99	3.38	3.28	0.97	3.60	3.60	1.00
2.30	2.47	1.07	2.60	2.72	1.05	0.07	0.02	0.29	0.12	0.05	0.42	0.37	0.25	0.68	0.47	0.35	0.74	1.48	1.24	0.84
1.68	1.38	0.82	1.80	1.57	0.87	12.25	9.72	0.79	12.15	9.55	0.79	9.87	7.58	0.77	7.48	5.72	0.76	8.32	6.64	0.80
1.13	1.45	1.28	1.37	1.47	1.07	4.65	4.56	0.98	4.92	4.63	0.94	4.92	4.87	0.99	4.45	4.37	0.98	5.62	5.52	0.98
3.58	2.78	0.78	2.68	2.67	1.00	0.31	0.39	1.26	0.30	0.37	1.23	0.53	0.67	1.26	0.55	0.68	1.24	1.54	1.76	1.14
1.17	1.30	1.11	1.23	1.35	1.10	5.86	5.94	1.01	5.95	5.97	1.00	.	.	.	4.55	4.63	1.02	4.32	4.62	1.07
1.15	0.87	0.76	1.30	1.07	0.82	4.80	5.14	1.07	4.73	5.05	1.07	4.40	5.10	1.16	3.77	4.10	1.09	3.88	4.26	1.10
1.10	1.23	1.12	1.00	1.12	1.12	17.60	15.45	0.88	16.55	13.78	0.83	12.33	10.12	0.82	7.82	6.57	0.84	6.42	5.76	0.90
2.32	1.65	0.71	2.00	1.55	0.77	.	.	.		.	.	41.13	30.82	0.75	22.17	16.92	0.76	15.62	12.32	0.79

actions in the retention of enantiomers relative to other equilibria, *e.g.*, complexation. The magnitude of the effect is expected to be more pronounced on packing 3 (*n*-octyl chains) than on short chain silicas, *e.g.*, packing 2. In this case, retention of hydrophobic α-amino acids should increase only slightly or even fall with increasing methanol content, while hydrophilic α-amino acids show an increase in k'. In fact, these predicted changes are seen in Table II for packing 3: for α-amino acids of low hydrophobic character, such as Asp, Glu, Ala, Asn, Ser, Thr, Lys and Arg, k' is enhanced by addition of methanol, indicating that complexation dominates for the reasons already discussed in ref. 14. For hydrophobic α-amino acids, such as Phe, Trp, Leu and Ile the opposite occured: k' diminished steadily as a result of the hydrophobic interactions dominating over complexation. In contrast to packing 3, on packing 2 the dependence of k' on the methanol content followed a much more uniform course for all α-amino acids. The retention first decreased to a minimum at a content of *ca.* 30 % (v/v) and then increased. Only the retention of His remained unaffected by the variation of methanol content. The course through a minimum indicates a complex behaviour which cannot yet be explained. The elution sequence did not change with the methanol content on packings 2 and 3, with the exception of Phe and Val on packing 2 and Thr and Asp on packing 3. The elution order of solutes on these two packings was identical, except for Leu, Arg, Met, Lys and Asn. These results reveal that complexation interactions are very sensitive not only to pH changes but also to changes of eluent polarity.

Type of organic solvent in the eluent (Table III). Three typical reversed-phase solvents, methanol (CH_3OH), acetonitrile (CH_3CN) and tetrahydrofuran (THF), were employed at a constant content of 50:50 (v/v). Similar to observations on reversed-

TABLE III

EFFECT OF TYPE OF ORGANIC SOLVENT IN THE ELUENT ON RETENTION AND SELECTIVITY OF α-AMINO ACID ENANTIOMERS

Conditions as in Table I; pH 5.0, organic–aqueous solvent (50:50, v/v).

Amino acid	*[Cu(L-hydroxyprolyl)methyl]$^+$ silica (Si 60) type of organic solvent*									*[Cu(L-hydroxyprolyl)methyl]$^+$ silica (Si 100) type of organic solvent*									*[Cu(L-hydroxyprolyl)n-octyl]$^+$ silica (Si 100) type of organic solvent*								
	THF			*CH_3CN*			*CH_3OH*			*THF*			*CH_3CN*			*CH_3OH*			*THF*			*CH_3CN*			*CH_3OH*		
	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α
Asp	1.52	1.44	0.95	3.40	3.36	0.99	2.64	2.52	0.95	.	.	.	0.47	0.55	1.17	0.55	0.62	1.13	4.90	5.30	1.08	6.85	7.37	1.07	10.70	10.58	0.99
Glu	1.25	1.46	1.17	2.92	3.27	1.12	2.04	2.33	1.14	.	.	.	0.55	0.75	1.36	0.58	0.82	1.41	2.45	2.43	0.99	5.05	5.17	1.02	6.42	5.47	0.85
His	2.65	1.42	0.54	5.86	3.46	0.59	4.42	2.02	0.46	0.52	0.37	0.71	1.83	1.37	0.75	1.70	1.13	0.67	3.23	2.98	0.92	3.22	6.28	1.95	2.77	7.85	2.83
Ala	1.47	1.61	1.09	2.68	2.95	1.10	2.10	2.34	1.11	0.20	0.33	1.65	0.86	1.18	1.37	0.80	1.00	1.25	0.87	0.87	1.00	1.67	1.95	1.17	2.00	1.68	0.84
Asn	2.37	2.07	0.87	4.87	4.47	0.92	4.03	3.40	0.84	0.10	0.30	3.00	0.76	1.27	1.67	0.93	1.33	1.43	1.80	1.53	0.83	1.85	1.50	0.81	2.12	1.82	0.86
Ser	1.83	1.73	0.95	3.59	3.32	0.93	3.00	2.65	0.88	0.25	0.25	1.00	0.98	0.95	0.97	0.97	1.00	1.03	1.87	1.87	1.00	2.47	2.27	0.92	2.55	2.38	0.93
Pro	1.74	2.81	1.61	2.61	4.25	1.63	2.33	3.92	1.68	0.23	0.52	2.26	0.82	1.52	1.85	0.85	1.78	2.09	0.93	1.78	1.92	1.92	3.83	2.00	1.97	4.18	2.12
Thr	2.08	2.32	1.12	4.00	4.42	1.11	3.41	3.51	1.03	0.28	0.52	1.86	1.05	1.55	1.48	1.18	1.62	1.37	2.15	1.92	0.89	2.58	2.38	0.92	2.92	2.62	0.90
Val	1.71	1.77	1.03	3.01	3.08	1.02	2.49	2.52	1.01	0.43	0.37	0.86	1.05	0.97	0.92	1.05	1.00	0.95	1.82	1.58	0.87	3.08	3.20	1.04	3.38	3.28	0.97
Lys	2.50	2.27	0.91	.	.	.	4.90	4.15	0.85	0.63	0.62	0.98	3.15	3.23	1.03	2.60	2.72	1.05	0.58	0.47	0.81	0.70	0.57	0.81	0.47	0.35	0.74
Tyr	2.04	1.72	0.85	3.12	2.64	0.85	3.13	2.43	0.78	1.08	0.98	0.91	1.65	1.43	0.87	1.80	1.57	0.87	2.85	2.03	0.71	5.67	4.37	0.77	7.48	5.72	0.76
Met	1.67	2.02	1.21	2.90	3.45	1.19	2.66	3.00	1.13	0.60	0.68	1.13	1.27	1.40	1.10	1.37	1.47	1.07	2.20	2.03	0.92	3.58	3.38	0.94	4.45	4.37	0.98
Arg	2.60	2.80	1.08	.	.	.	4.42	4.64	1.05	0.78	0.75	0.96	3.13	3.15	0.99	2.68	2.67	1.00	0.67	0.77	1.14	0.87	0.97	1.11	0.55	0.68	1.24
Ile	1.52	1.48	0.98	2.58	2.49	0.96	2.20	2.13	0.97	0.60	0.72	1.20	1.20	1.38	1.15	1.23	1.35	1.10	2.33	2.02	0.87	4.30	4.47	1.04	4.55	4.63	1.02
Leu	1.75	1.56	0.89	2.92	2.62	0.90	2.56	2.30	0.90	0.70	0.48	0.68	1.28	1.03	0.81	1.30	1.07	0.82	1.60	1.65	1.03	3.28	3.68	1.12	3.77	4.10	1.09
Phe	1.96	1.86	0.95	2.87	2.69	0.94	3.04	2.65	0.87	0.40	0.63	1.58	0.68	0.90	1.32	1.00	1.12	1.12	3.02	2.25	0.74	5.45	4.70	0.86	7.82	6.57	0.84
Trp	3.05	2.39	0.78	4.28	3.02	0.71	5.27	3.47	0.66	1.32	0.95	0.72	1.33	1.18	0.89	2.00	1.55	0.77	5.00	3.63	0.73	11.70	9.25	0.79	22.17	16.92	0.76

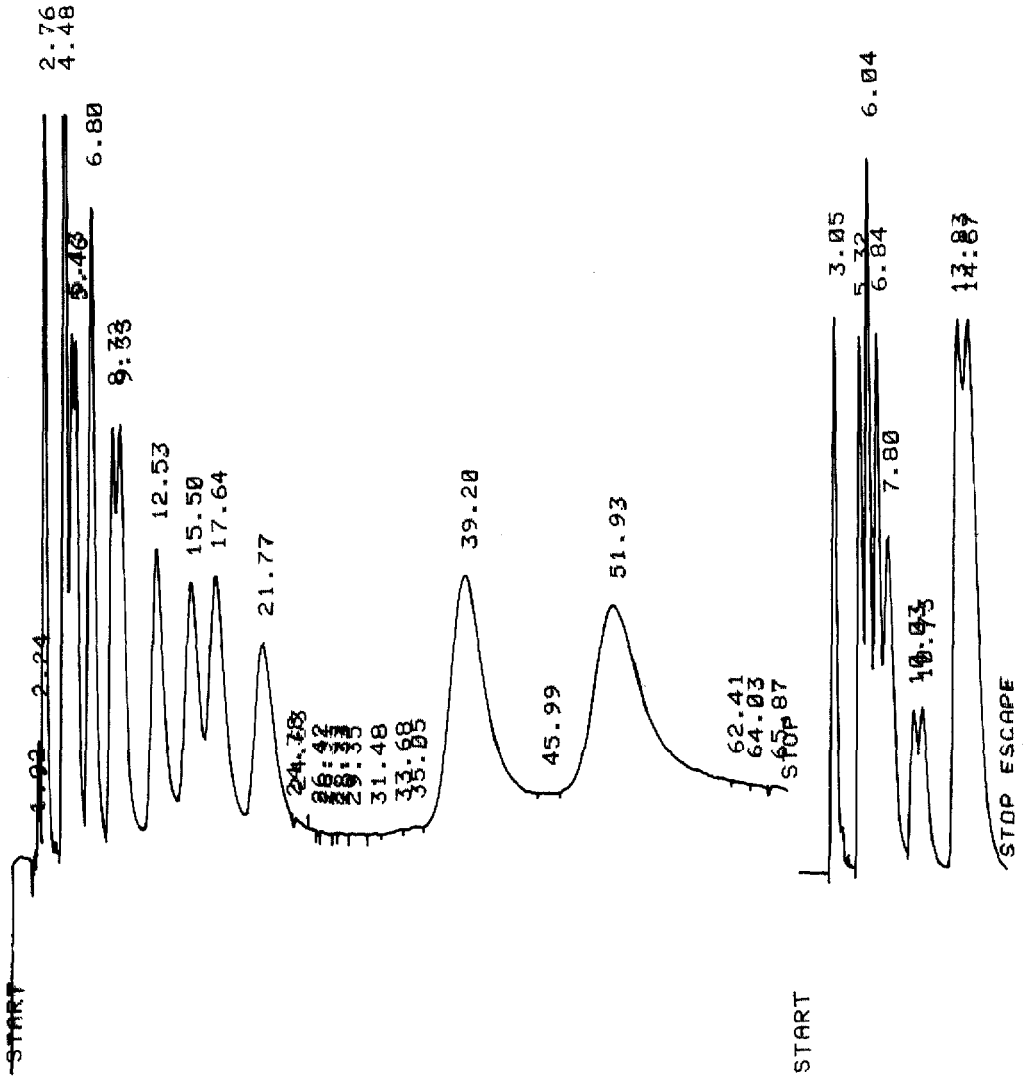

Fig. 2. Separation of six racemic α-amino acids into their enantiomers on (L-hydroxyprolyl)*n*-octyl silica, d_p = 10 μm. Column, 100 × 4.6 mm; eluent, 10^{-4} *M* copper acetate + 0.01 *M* ammonium acetate (pH 4.8) in methanol–water (70:30, v/v); column temperature, 323°K; flow-rate, 1.5 ml/min; UV detector, 254 nm. Elution order, DL-Arg, L-Pro, D-Ser, L-Ser, D-Pro, D-Gln, L-Gln, D-Tyr, L-Tyr, D-Phe, L-Phe, D-Trp, L-Trp.

Fig. 3. Separation of four racemic α-amino acids. Conditions as in Fig. 2. Elution order, DL-Lys, L-His, D-Asn, L-Asn, D-His, D-Glu, L-Glu, L-Asp, D-Asp.

phase packings, on packing 3 the elution order followed the sequence $k'_{CH_3OH\text{–aqueous eluent}} > k'_{CH_3CN\text{–aqueous eluent}} > k'_{THF\text{–aqueous eluent}}$, (except for basic α-amino acids), whereas on packing 2 the sequence was more complicated and comparable to that obtained on (L-hydroxyprolyl)propyl silica. In agreement with the previous ob-

TABLE IV

EFFECT OF COLUMN TEMPERATURE ON RETENTION AND ENANTIOSELECTIVITY

Columns, packings and eluent as in Table I.

Amino acid	*[Cu(L-hydroxyprolyl)methyl]$^+$ silica (Si 60) column temperature (°K)*									*[Cu(L-hydroxyprolyl)methyl]$^+$ silica (Si 100) column temperature (°K)*									*[Cu(L-hydroxyprolyl)n-octyl]$^+$ silica (Si 100) column temperature (°K)*								
	298			*323*			*348*			*298*			*323*			*348*			*298*			*323*			*348*		
	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α	k'_L	k'_D	α
Asp	1.92	2.10	1.09	1.10	1.03	0.94	0.86	0.81	0.94	0.17	0.17	1.00	0.05	0.05	1.00	0.05	0.05	1.00	1.75	1.85	1.06	2.01	2.13	1.06	1.90	2.02	1.06
Glu	1.71	1.84	1.08	1.02	1.13	1.11	0.84	0.83	0.99	0.17	0.22	1.29	0.05	0.13	2.60	0.18	0.15	0.87	1.25	1.33	1.06	1.57	1.57	1.00	1.57	1.57	1.00
His	4.13	2.34	0.57	3.80	2.33	0.61	4.01	2.52	0.63	1.83	1.45	0.79	1.97	1.78	0.90	2.15	1.98	0.92	2.43	1.47	0.60	2.22	1.92	0.86	2.77	1.85	0.67
Ala	1.26	1.39	1.10	1.40	1.47	1.05	1.46	1.53	1.05	0.48	0.60	1.25	0.50	0.58	1.16	0.60	0.65	1.08	0.33	0.42	1.27	0.40	0.58	1.45	0.63	0.83	1.32
Asn	2.39	2.18	0.91	2.05	1.76	0.86	1.47	1.50	1.02	1.10	1.10	1.00	0.41	0.57	1.39	0.33	0.60	1.82	0.85	0.80	0.94	0.89	0.82	0.92	1.03	1.03	1.00
Ser	1.92	1.61	0.84	1.72	1.46	0.85	1.63	1.32	0.81	0.45	0.45	1.00	0.45	0.45	1.00	0.50	0.50	1.00	0.70	0.60	0.86	0.82	0.74	0.90	1.08	0.98	0.91
Pro	1.99	2.67	1.34	1.93	2.51	1.30	1.70	2.27	1.33	1.07	1.57	1.47	1.05	1.47	1.40	1.15	1.28	1.11	0.90	1.80	2.00	1.07	2.06	1.93	1.08	2.22	2.05
Thr	2.57	2.18	0.85	2.14	1.94	0.91	2.10	1.94	0.92	1.06	1.13	1.07	0.57	0.65	1.14	0.62	0.68	1.10	1.10	1.07	0.97	1.14	1.16	1.02	1.65	1.62	0.98
Val	3.30	2.08	0.63	2.10	2.12	1.01	2.17	2.32	1.07	.	.	.	1.42	1.18	0.83	1.58	1.23	0.78	2.17	1.97	0.91	2.81	2.71	0.96	3.65	3.58	0.98
Lys	2.01	2.01	1.00	2.77	2.61	0.94	3.72	3.47	0.93	2.57	2.75	1.07	2.82	2.72	0.96	3.70	3.60	0.97	0.03	0.03	1.00	.	.	0.29	0.28	0.25	0.89
Tyr	2.64	1.74	0.66	2.30	1.52	0.66	2.13	1.51	0.71	1.07	0.68	0.64	1.01	0.80	0.79	0.83	0.72	0.87	11.78	10.37	0.88	12.25	9.72	0.79	11.98	9.90	0.83
Met	3.07	3.32	1.08	2.87	2.82	0.98	2.38	2.51	1.05	1.33	1.48	1.11	1.53	1.53	1.00	1.23	1.63	1.33	4.32	4.25	0.98	4.0	4.56	1.13	5.32	5.25	0.99
Arg	2.75	2.87	1.04	3.40	3.44	1.01	4.26	4.26	1.00	4.48	4.58	1.02	3.53	3.37	0.95	4.28	4.12	0.96	0.35	0.43	1.23	0.31	0.39	1.26	0.70	0.83	1.18
Ile	2.87	3.04	1.06	2.45	2.51	1.02	2.40	2.43	1.01	1.48	1.57	1.06	.	.	.	1.72	1.70	0.99	4.65	4.30	0.92	5.86	5.94	1.01	7.22	7.32	1.01
Leu	2.92	3.47	1.19	2.83	3.02	1.07	2.62	2.51	0.96	1.43	1.25	0.87	1.67	1.45	0.87	1.67	1.28	0.77	3.77	3.97	1.05	4.80	5.14	1.07	6.00	6.53	1.09
Phe	4.17	3.66	0.88	3.35	2.87	0.86	2.92	2.57	0.88	.	.	.	1.58	1.78	1.13	1.55	1.55	1.00	17.70	14.33	0.81	17.60	15.45	0.88	18.53	16.60	0.90
Trp	6.67	3.36	0.50	4.69	2.69	0.57	3.70	2.66	0.72	2.17	1.28	0.59	.	.	.	1.53	0.98	0.64	.	.	.	.	.	.	65.66	55.13	0.84

servations (Table II), the hydrophobic interactions on packing 3 were much more pronounced than on packing 2.

By substituting CH_3OH by CH_3CN and THF, the elution sequence remained unchanged on packing 2, whereas on packing 3, Ile, Val, Ala and His behaved differently.

Concentration of ammonium acetate in the eluent. As previously stated[14] the capacity factor of α-amino acids decreased with the concentration of ammonium acetate in the range studied. This is the result of increasing competition of the eluent and solute at the fixed ligand sites. No noticeable enantioselectivity developed on varying the ammonium acetate concentration.

Column temperature (Table IV). Column temperature, T_c, appears to be an important parameter in the separation of enantiomers for several reasons: (i) All variations increase, decrease, no change of k' were observed when raising T_c from 293 to 348°K. This resulted in some useful selectivity differences. In some cases a reversal of the elution sequence took place with increasing T_c. The specific course of the plot k' *vs.* T_c indicated that the temperature affects individual interactions in "syndromic" (synergistic) manner. (ii) A noticeable improvement of column efficiency was obtained by raising T_c; this resulted in a higher resolution. For that reason most of the studies were carried out at 323°K.

In conclusion, column temperature plays a much more significant rôle in ligand-exchange chromatography than in adsorption chromatography.

CONCLUSIONS

Enantiomer separations on (L-hydroxyprolyl)alkyl silicas (Figs. 2 and 3) exhibit the following advantages: (i) uncomplicated eluents; (ii) ease of detection by means of a common UV photometer at 254 nm; (iii) high peak capacity of columns (eight pairs of α-amino acids may be resolved); (iv) ease of control of retention by appropriate manipulation of eluent pH and methanol content; (v) excellent enantioselectivity. Further benefits of this method are the possibility of scale-up to preparative separations and application to the separation of other racemic compounds. Although operating most favourably in a pH range between 4 and 6, the column packings should be further tested with respect to their chemical stability and batch-to-batch reproducibility. The detailed examination of the complex surface structure of these packings, in order to determine the individual types of interactions contributing to retention, promises to be of significant interest.

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