*Journal of Chromatography*, 260 (1983) 75–79
Elsevier Science Publishers B.V., Amsterdam — Printed in the Netherlands

CHROM. 15,599

# ENANTIOMERIC RESOLUTION OF AMINO ACIDS BY HIGH-PERFORMANCE LIGAND-EXCHANGE CHROMATOGRAPHY USING HISTIDINE-BONDED SILICA GEL

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(Received December 7th, 1982)

## SUMMARY

The preparation of histidine-bonded silica gel is described. The formation of an amide bond between aminopropyl silica gel and Boc-Histidine ($^{im}$Tos) and cleavages of the protecting groups were characterized by IR spectroscopy. This histidine-bonded silica gel was able to resolve enantiomers of many amino acids, irrespective of the type of amino acids. In addition, D,L-mandelic acid was well resolved.

## INTRODUCTION

The usefulness of ligand-exchange liquid chromatography using $Cu^{2+}$–amino acid complexation for chiral resolution of amino acids is well documented[1–16]. Synthetic polymer gels such as polystyrene and amide gel were used as the base matrix of amino acid bonding in most of the studies. Hence, in most of such work high-performance liquid chromatography (HPLC) was not employed. On the other hand, the application of small-size silica gel is a comparatively new area, with great potential for use in HPLC. Not many kinds of amino acid bonded to silica gel have been used so far or amino acids resolved[6,10–12,17]. L-Proline, which was successfully used as a counterpart of the synthetic polymer phase, has been bonded on Partisil-5. The resolution of only three amino acids (tryptophan, phenylalanine and tyrosine) by this proline phase has been reported[6]. L-Phenylalanine-bonded silica gel resolved D,L-histidine completely and D,L-tryptophan partly[17]. L-Tryptophan- and L-valine-bonded silica gels resolved D,L-histidine very well[17,18].

In general, L-amino acid-bonded polymer phases retain L-amino acids in preference to D-species, with the exception of reversed pairing between three aromatic amino acids (tryptophan, phenylalanine and tyrosine) and other amino acids. It seems that the pairing of bonded and resolved amino acids is preserved on the silica gel phase, suggesting minor participation of the silica gel surface itself in chiral recognition. These results indicate that histidine-bonded silica gel might be able to resolve enantiomers. This paper describes the derivatization of silica gel with L-histidine and the enantiomeric resolution of amino acids and other compounds.

0021-9673/83/$03.00 

## EXPERIMENTAL

### *Preparation of L-histidine-bonded silica gel*

The method via the active ester with N-hydroxysuccinimide previously reported[17] failed with N-*tert.*-butyloxycarbonyl-$N^{imidazole}$-tosyl-L-histidine [Boc-His(Tos)]. Coupling via the active N-acyl group by reaction with 1,1'-carbonyldiimidazole (CDI) was succesfully tried.

The silica gel used was Develosil 100/10 (Nomura Chemical, Aichi, Japan) of mean diameter 10 $\mu$m, mean pore size 10 nm and surface area 350 $m^2/g$. Aminopropyl silica gel was prepared according to the procedure described previously[17]. Boc-His(Tos) (Peptide Institute, Osaka, Japan) was added to a dimethylformamide (DMF) (dried over molecular sieve) solution of CDI (Aldrich, Milwaukee, WI, U.S.A.) with stirring at room temperature, then the reaction vessel was cooled in an ice–water bath. A slight excess of Boc-His(Tos) over CDI was used. After evolution of carbon dioxide had ceased, an amount of aminopropyl silica gel corresponding to the equivalent amino group to CDI used was added to the reaction vessel. The suspension was gently stirred for 24 h at ambient temperature. The modified silica gel was thoroughly washed successively with DMF, methanol and dichloromethane and dried under vacuum.

### *Characterization of bonded phase*

The degrees of bonding of the aminopropyl function and Boc-His(Tos) were determined as 0.76 and 0.36 mmole/g, respectively, by Kjeldahl nitrogen analysis. The bonding of Boc-His(Tos) was also evaluated as 0.38 mmole/g from the increase in weight of the modified silica gel. Both results were consistent. It was shown by application to other Boc-amino acids that this coupling method via the active N-acyl group provided a versatile and more convenient method than with that via the active ester with N-hydroxysuccinimide.

The Boc group was deprotected by treatment with 30% trifluoroacetic acid in dichloromethane (freshly distilled over calcium hydride). The end of deprotection was determined by watching the evolution of gas through a capillary tube immersed in methanol. Deprotection of the tosyl group was achieved with treatment by hydroxybenzotriazole in THF for 2 h. The modified silica gels were characterized by IR spectroscopy. The appearance of amide bands in the range 1700–1500 $cm^{-1}$ confirmed the formation of an amide bond between aminopropyl silica gel and Boc-His(Tos)[17]. These amide bands remained even after both deprotection steps, as expected. The cleavage of the Boc group was ascertained by the disappearance of the doublet at 1365–1395 $cm^{-1}$ characteristic of the *tert.*-butyl group[17]. Complete deprotection of the tosyl group was confirmed by the disappearance of the peaks at 703, 676, 591 and 542 $cm^{-1}$.

### *Column and HPLC*

Treatment with $Cu^{2+}$ solution made histidine-bonded silica gel blue following the two deprotection steps. The modified silica gel was packed into stainless-steel columns (10 cm × 2 mm I.D.) using a balanced slurry prepared from tetrabromoethane, carbon tetrachloride and methanol. $Cu^{2+}$ was loaded by passing the eluent containing $Cu^{2+}$ after the packing and washing with methanol and water. The HPLC ap-

paratus has been described previously[16]. The eluent was 1/15 *M* phosphate buffer prepared from distilled water, adjusted to the appropriate pH and containing $10^{-4}$ *M* copper ion.

## RESULTS AND DISCUSSION

Histidine-bonded silica gel (His-Sil) resolved enantiomers of many amino acids, irrespective of the type of amino acid, involving acidic, basic, hydrophilic, hydrophobic and aromatic amino acids. This versatility of His-Sil is in clear contrast to the properties of other amino acid-modified silica gels. Table I shows the capacity

TABLE I

CAPACITY RATIO ($k'$) AND RESOLUTION FACTOR ($\alpha_{L/D}$) FOR AMINO ACIDS RESOLVED BY HIS-SIL

Eluent: 1/15 *M* phosphate buffer–$10^{-4}$ *M* $Cu^{2+}$, pH 4.56.

| *Compound* | *Enantiomer* | $k'$ | $\alpha_{L/D}$ |
|---|---|---|---|
| Alanine | L | 1.49 | 1.10 |
| | D | 1.35 | |
| Valine | L | 2.22 | 1.18 |
| | D | 1.88 | |
| Leucine | L | 2.34 | 1.12 |
| | D | 2.09 | |
| Threonine | L | 4.65 | 1.10 |
| | D | 4.22 | |
| Asparagine | L | 5.50 | 0.96 |
| | D | 5.74 | |
| Serine | L | 3.19 | 1.06 |
| | D | 3.01 | |
| Glutamine | L | 2.77 | 1.12 |
| | D | 2.48 | |
| Aspartic acid | L | 19.0 | 0.97 |
| | D | 19.5 | |
| Glutamic acid | L | 13.0 | 1.17 |
| | D | 11.1 | |
| Histidine | L | 4.21 | 1.10 |
| | D | 3.84 | |
| Phenylalanine | L | 6.41 | 0.87 |
| | D | 7.40 | |
| Tyrosine | L | 6.51 | 0.72 |
| | D | 9.03 | |
| Tryptophan | L | 14.4 | 0.56 |
| | D | 25.5 | |
| Methionine | L | 3.94 | 1.04 |
| | D | 3.79 | |
| Arginine | L | 0.86 | 1.18 |
| | D | 0.73 | |
| DOPA | L | 8.12 | 0.67 |
| | D | 12.2 | |
| Mandelic acid | L | 5.57 | 1.08 |
| | D | 5.13 | |

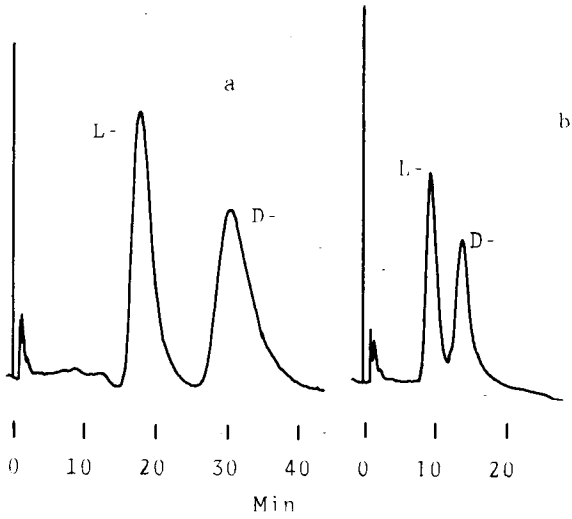


Fig. 1. Examples of enantiomeric resolution. Column, one His-Sil (10 cm × 2 mm I.D.); eluent, 1/15 *M* phosphate buffer–$10^{-4}$ *M* $Cu^{2+}$, pH 4.56; flow-rate, 0.37 ml/min; UV detection, 210 nm; (a) 0.04 and (b) 0.08 a.u.f.s. (a) D,L-Tryptophan; (b) D,L-DOPA.

ratios ($k'$) and resolution factors [$\alpha_{L/D}(=k'_L/k'_D)$] of the amino acids resolved, including mandelic acid as a non-amino acid.

Chromatograms of authentic mixtures of enantiomers are shown in Figs. 1 and 2. D,L-Tryptophan and D,L-DOPA (3,4-dihydroxyphenylalanine or 3-hydroxy-

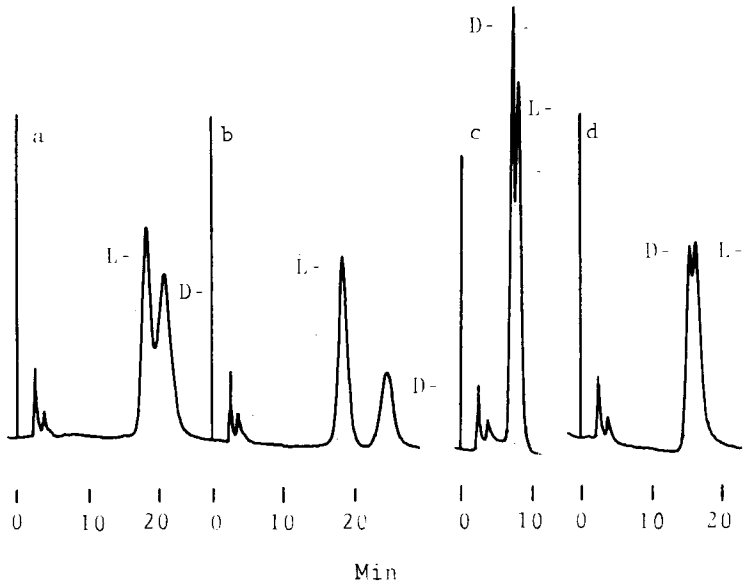


Fig. 2. Examples of enantiomeric resolution. Columns, three His-Sil (10 cm × 2 mm I.D.); conditions as in Fig. 1; 0.08 a.u.f.s. (a) D,L-Phenylalanine; (b) D,L-tyrosine; (c) D,L-valine; (d) D,L-mandelic acid.

tyrosine) can be completely resolved even by a single column. Mandelic acid was expected to participate in chelate formation with copper ion using hydroxyl and carboxyl groups, as shown by the resolution of the enantiomers. D,L-Mandelic acid can be partly resolved, but by three columns connected in series, as shown in Fig. 2d. The resolution of D,L-tyrosine is particularly remarkable.

The pH dependence of the retention of amino acids with His-Sil was similar to that in the previous study of the synthetic polymer phase[16], that is a rapid increase in the retention with increase in pH. On the other hand, mandelic acid showed an opposite dependence to those observed with amino acids.

L-Moieties of most amino acids (except aromatic ones) were retained more strongly than the corresponding D-moieties. Thus, pairing of L–L prevails in His-Sil also. The results correspond with the fact that Cu(L-His)$_2$ is preferred, in terms of the enthalpy of formation, to the *meso*-complex Cu(L-His) (D-His) in the copper–histidine bis-complex[19], although it was reported that stereoselectivity was negligible in the formation constants of the Cu(His)$_2$ bis-complex[20,21]. In the copper bis-complex involving histidine, the histidine residue functions as a tridentate chelate with a distorted apical carboxyl group, resulting in a distorted octahedral configuration[22]. An amide carbonyl group in the bonded phase also may participate in the chelate formation in a manner similar to that in solution or crystals. Hence it is likely that an additional distortion may contribute to the chiral recognition for many amino acids.

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