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Mechanistic evaluation of the resolution of α-amino acids on dynamic chiral stationary phases derived from amino alcohols by ligand-exchange chromatography

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Abstract

Two dynamic chiral stationary phases (CSP 7 and 12) were prepared starting from (R)-2-amino-1-propanol and (R)-α-phenylethylamine. The resolution of racemic α-amino acids on the two dynamic CSPs thus prepared were compared with previously reported resolution data on a dynamic CSP (2) derived from (1S,2R)-norephedrine. The enantioselectivity for the two enantiomers of α-amino acids on CSP 7 was found to be comparable to or better than that on CSP 2 while the enantioselectivity for the two enantiomers of α-amino acids on CSP 12 was much worse than that on CSPs 2 or 7. From these results, we concluded that the phenyl functionality at the first chiral center of CSP 2 is not essential in the chiral recognition. However, the hydroxy functionality of CSPs 2 or 7 does play a very important role in the chiral recognition.

1. Introduction

Chiral ligand-exchange chromatography has been widely employed in resolving racemic α-amino acids since the pioneering work of Davankov and co-workers in the late 1960s (see [1–3]). Copper(II) complexes of optically pure α-amino acids and their derivatives have been usually applied as chiral mobile phase additives [4,5] or chiral stationary phases (CSPs) after binding covalently [6,7] or hydrophobically [8–10] to solid column support. However, copper(II) complexes of other optically active materials have been rarely adopted as chiral selectors in chiral ligand-exchange chromatography [1,11,12].

Recently, we reported the use of the Cu(II)

complex of a (1S,2R)-norephedrine derivative **1** mechanically adsorbed onto a commercial octadecyl-silica gel column as a dynamic chiral stationary phase (CSP 2) in resolving underivatized racemic α-amino acids [13,14]. CSP 2 was very efficient in resolving various underivatized α-amino acids. Based on the resolution trends influenced by the organic modifier content, the Cu(II) concentration and the pH of the mobile phase, a chiral recognition model demonstrating the formation of the energetically different two diastereomeric ternary complexes shown in Fig. 1 from the fixed chiral ligand, racemic amino acid and Cu(II) was proposed [14]. In that model, chiral selector is bound to octadecyl-silica gel through the lipophilic interaction between the octadecyl chains of silica gel and the dodecyl alkyl chain of the chiral selector. The bounded
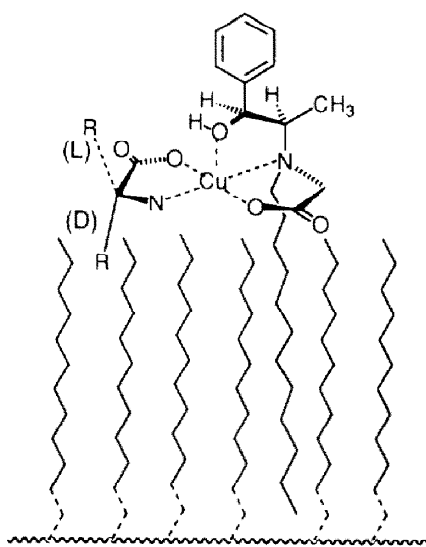
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Fig. 1. The proposed structure of the ternary complex formed from the fixed ligand of CSP 2, D- or L-amino acid and Cu(II).

chiral selector and an α-amino acid coordinate Cu(II), forming a ternary complex with *trans* (N,N) arrangement which is known to be energetically more favorable than that with *cis* (N,N) arrangement [8]. Simultaneously, the hydroxy oxygen of the chiral selector occupies the axial position in the coordination sphere of the Cu(II) of the square planar complex. However, based on the model shown in Fig. 1, the phenyl functionality at the first (1S)-chiral center of the chiral selector does not seem to have any role in the chiral recognition except hindering the axial coordination by the hydroxy oxygen because of the steric hindrance.

To explore the role of the phenyl functionality and the importance of the axial coordination by the hydroxy group in the chiral recognition model shown in Fig. 1, in the present study, we wish to prepare two new dynamic CSPs, one of which does not contain a phenyl functionality and the other of which does not have an axially coordinating hydroxy group. The resolution of α-amino acids on the two new dynamic CSPs may elucidate the role of the phenyl group and the importance of the axial coordination by the hydroxy group in the chiral recognition.

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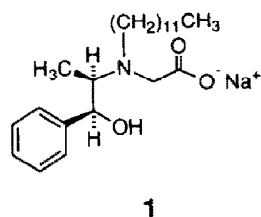
2. Experimental

2.1. Instrumentation

Melting points were determined by a Rigaku thermal analyzer TAS 100. ^{1}H NMR spectra were recorded on a Varian Gemini 300 or on a Varian Gemini 200 spectrometer using tetramethylsilane as an internal standard. IR spectra were recorded on a Mattson Polaris or Mattson Galaxy 2000 Fourier transform IR spectrometer.

Chromatography was performed with a Waters Model 510 pump, a Waters Model U6K universal chromatographic injector equipped with a 20-μl sample loop, a Waters Model 441 absorbance detector with 254-nm UV filter, and a Waters Model 740 data module recorder.

2.2. Preparation of dynamic CSP 7 from (R)-2-amino-1-propanol

Dynamic CSP **7** based on (R)-2-amino-1-propanol was prepared as shown in Fig. 2. The detailed procedures are as follows.

(R)-N-Lauroyl-2-amino-1-propanol 3

In a 250-ml flask equipped with a magnetic stir bar were dissolved 15 ml (0.064 mol) of (R)-2-amino-1-propanol and 10 ml (0.072 mol) of triethylamine in 50 ml of methylene chloride. To the stirred mixture were slowly added 15 ml (0.064 mol) of lauroyl chloride dissolved in 20 ml of methylene chloride under nitrogen. The reaction mixture was stirred at room temperature for 2 h and then washed with saturated NaHCO$_3$ solution three times. The organic solution was dried over anhydrous MgSO$_4$ and the solvent was evaporated. The solid residue was crys-
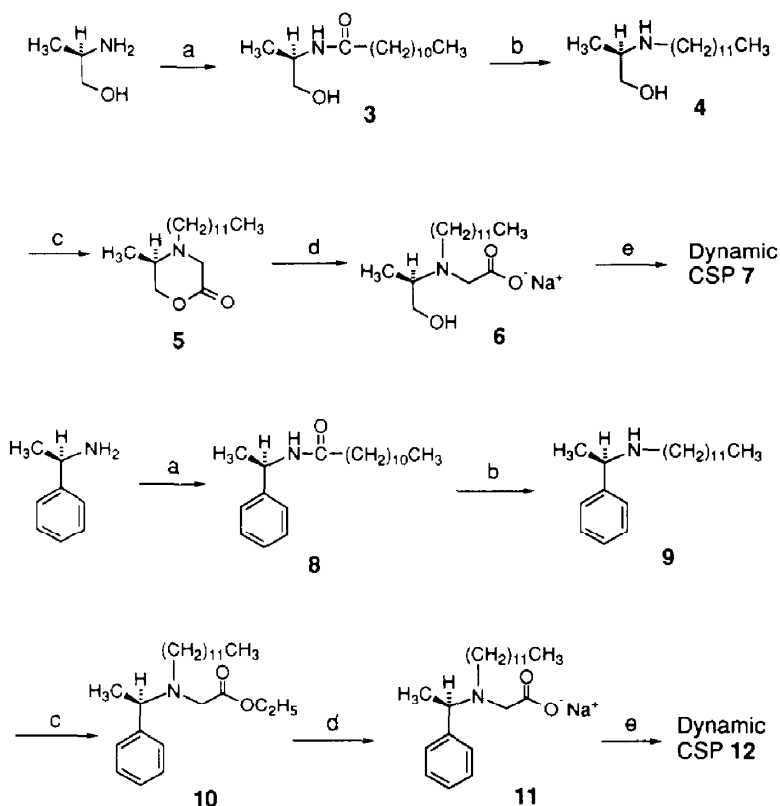
Fig. 2. Preparation of CSPs 7 and 12. a = Lauroyl chloride, triethylamine, CH_2Cl_2, room temperature; b = $LiAlH_4$, THF, 0°C to reflux; c = ethylbromoacetate, propylene oxide, CH_2Cl_2, room temperature; d = 1 M NaOH in methanol; e = hydrophobic loading onto a commercial octadecyl-silica gel column.

tallized from the mixed solvent of acetone and hexane to afford compound 3 as a white crystalline solid (13.5 g, 80% yield). m.p. 70–72°C; ^{1}H NMR (C^2HCl_3) δ 0.87 (t, 3H), 1.16 (d, 3H), 1.25 (broad s, 16H), 1.55–1.70 (m, 2H), 2.18 (t, 2H), 3.08 (broad s, 1H), 3.48–3.69 (m, 2H), 4.00–4.12 (m, 1H), 5.68 (broad s, 1H). IR (KBr) cm^{-1} 3302, 2922, 2852, 1641, 1549, 1467.

(R)-N-Dodecyl-2-amino-1-propanol 4

In a 250-ml flask charged with 10 g (0.038 mol) of compound 3 and 6 g of $LiAlH_4$ were added 50 ml of dry tetrahydrofuran (THF) at 0°C under nitrogen. The mixture was heated to reflux for one day. The reaction mixture was cooled to 0°C and then the reaction was quenched by adding water. The whole mixture was filtered through a

Celite pad and then THF was removed under reduced pressure. The aqueous solution was extracted with methylene chloride, and then the organic layer was dried over anhydrous $MgSO_4$ and concentrated. The resulting residue was crystallized from the mixed solvent of methylene chloride and hexane to afford compound 4 as a white crystalline solid (7.6 g, 83% yield). m.p. 63–65°C; ^{1}H NMR (C^2HCl_3) δ 0.88 (t, 3H), 1.10 (d, 3H), 1.25 (broad s, 18H), 1.47–1.56 (m, 2H), 2.49–2.62 (m, 1H), 2.68–2.88 (m, 4H; 2H with ^2H_2O), 3.32 (dd, 1H), 3.62 (dd, 1H). IR (KBr) cm^{-1} 2919, 2849, 1466.

(R)-4-Dodecyl-5-methyl-2,3,5,6-tetrahydro-4H-1,4-oxazin-2-one 5

In a 250-ml flask equipped with a magnetic stir bar were dissolved 6 g (0.025 mol) of compound

4 in 50 ml of methylene chloride. To the stirred solution were added 2.75 ml (0.025 mol) of ethylbromoacetate and 1 ml of propylene oxide at room temperature. The whole mixture was stirred for one day at room temperature and then washed with water. The organic solution was dried over anhydrous $MgSO_4$, filtered, concentrated and the resulting residue was purified by silica gel column chromatography to afford product 5 as a sticky solid (2.2 g, 27% yield). ^1H NMR (C^2HCl_3) δ 0.88 (t, 3H), 1.08 (d, 3H), 1.26 (broad s, 18H), 1.39–1.47 (m, 2H), 2.24–2.32 (m, 1H), 2.60–2.69 (m, 1H), 2.77–2.83 (m, 1H), 3.19 (d, 1H), 3.60 (d, 1H), 4.05 (dd, 1H), 4.30 (dd, 1H); IR (C^2HCl_3) cm^{-1} 2928, 2857, 1740.

(R)-N,N-Carboxymethyl dodecyl-2-amino-1-propanol monosodium salt 6

Compound 6 (2.0 g, 0.006 mol) was dissolved in 20 ml of methanol in a 50-ml flask. To the stirred solution were added 6.2 ml of 1 M NaOH solution in methanol. The whole mixture was stirred for 5 h and then evaporated. The residue was dried under high vacuum for 10 h to afford oily product 6 (1.8 g). ^1H NMR (^2H_2O) δ 0.80–0.98 (broad m, 6H), 1.27 (broad s, 18H), 1.48 (broad s, 2H), 2.39 (broad s, 1H), 2.54 (broad s, 1H), 2.92 (broad s, 1H), 3.00 (broad d, 1H), 3.27 (broad d, 1H), 3.40 (broad s, 2H); IR (KBr) cm^{-1} 2900, 1620, 1480, 1410.

Preparation of dynamic CSP 7

Dynamic CSP 7 was prepared by loading 6 onto a octadecyl-silica gel column. The loading of 6 onto a commercial octadecyl-silica gel column (Waters μBondapak C_{18}, 300 × 3.9 mm) was accomplished at room temperature by eluting a solution of 6 (1.8 g, 0.0054 mol) in 50 ml of methanol–water (1:2, v/v) through the column (flow-rate: 0.8 ml/min) followed by washing with mixture of methanol–water (1:2, v/v, flow-rate: 0.3 ml/min) for 7 h and then with water (flow-rate: 0.3 ml/min) for 5 h. The effort to figure out the loaded amount of 6 was not successful. However, the used amount of 6 (1.8 g) was assumed to be large enough for full loading

because the bleeding of the excess of 6 from the column was detected by the UV monitor.

2.3. Preparation of dynamic CSP 12 from (R)-α-phenylethylamine

Dynamic CSP 12 based on (R)-α-phenylethylamine was prepared as shown in Fig. 2. The detailed procedures are as follows.

(R)-N-Lauroyl-α-phenylethylamine 8

This compound was prepared as a white solid material using 3.2 ml (0.025 mol) of (R)-(+)-α-phenylethylamine, 3.5 ml (0.025 mol) of triethylamine and 5.8 ml (0.025 mol) of lauroyl chloride by the same procedure as described in the preparation of compound 3 (7.13 g, 94% yield). m.p. 54.5–55.5°C; ^1H NMR (C^2HCl_3) δ 0.88 (t, 3H), 1.25 (broad s, 16H), 1.49 (d, 3H), 1.58–1.65 (m, 2H), 2.17 (t, 2H), 5.10–5.20 (m, 1H), 5.66 (d, 1H), 7.24–7.38 (m, 5H); IR (C^2HCl_3) cm^{-1} 3293, 3065, 2926, 2855, 1642.

(R)-N-Dodecyl-α-phenylethylamine 9

This compound was prepared as a yellowish liquid using 7.13 g (0.023 mol) of compound 8 and 1.78 g (0.05 mol) of $LiAlH_4$ by the same procedure as described in the preparation of compound 4 (5.77 g, 85% yield). ^1H NMR (C^2HCl_3) δ 0.88 (t, 3H), 1.18–1.30 (m, 19H), 1.37 (d, 3H), 1.42–1.52 (m, 2H), 2.37–2.54 (m, 2H), 3.77 (q, 1H), 7.27–7.36 (m, 5H); IR (C^2HCl_3) cm^{-1} 3065, 2926, 2855, 1466.

(R)-N-Ethoxycarbonylmethyl-N-dodecyl-α-phenylethylamine 10

This compound was prepared as an oily liquid using 5.79 g (0.02 mol) of compound 9, 2.22 ml (0.02 mol) of ethylbromoacetate and 2.78 ml (0.02 mol) of triethylamine by the same procedure as described in the preparation of compound 5 (4.66 g, 62% yield). ^1H NMR (C^2HCl_3) δ 0.89 (t, 3H), 1.24–1.33 (m, 21H, triplet for 3H is imbedded), 1.35 (d, 3H), 1.40–1.47 (m, 2H), 2.54–2.60 (m, 2H), 3.27 (d, 1H), 3.43 (d, 1H), 4.04 (q, 1H), 4.14 (q, 2H), 7.20–7.40 (m, 5H); IR (C^2HCl_3) cm^{-1} 3065, 2926, 2855, 1740.

(R)-N-Carboxymethyl-N-dodecyl-α-phenylethylamine mono sodium salt 11

This salt was prepared as an oily product using 4.66 g (0.012 mol) of compound **10** by the same procedure as described in the preparation of salt **6** (4.4 g). ^{1}H NMR (^{2}H$_2$O) δ 0.87 (t, 3H), 1.10–1.40 (m, 23H), 2.12–2.38 (broad m, 2H), 2.88 (d, 1H), 3.19 (d, 1H), 3.82–3.90 (m, 1H), 6.98–7.30 (m, 5H); IR (KBr) cm^{-1} 3100, 2950, 2870, 1650.

Preparation of dynamic CSP 12

Dynamic CSP **12** was prepared by loading **11** onto a octadecyl-silica gel column. The loading of **11** onto a commercial octadecyl-silica gel column (Waters μBondapak C$_{18}$, 300 × 3.9 mm) was achieved at room temperature by passing a solution of **11** (2.1 g, 0.0054 mol) in 50 ml of methanol–water (1:2, v/v) through the column (flow-rate: 0.8 ml/min) followed by washing with mixture of methanol–water (1:2, v/v; flow-rate: 0.3 ml/min) for 7 h and then with water (flow-rate: 0.3 ml/min) for 5 h. The effort to figure out the loaded amount of **11** was not successful. However, the used amount of **11** (2.1 g) was assumed to be large enough for full loading because the bleeding of the excess of **11** from the column was detected by the UV monitor.

2.4. Chromatography

To resolve racemic α-amino acids on the two dynamic CSPs prepared as above, a mobile phase was passed through the column until the baseline (UV monitor, 254 nm) became stable to equilibrate the column and then, a methanolic solution (usually 5 μl) containing a racemic or optically enriched α-amino acid was injected with a flow-rate of 0.8 ml/min. Mobile phase was prepared by dissolving a specified amount of CuSO$_4$ in deionized water or in deionized water containing acetonitrile or methanol as an organic modifier. Column void volume (the elution time of an unretained solute) was measured by injecting aqueous NaNO$_3$ solution [15]. The dynamic CSPs used in this study were found to be equally effective for the chiral separation of α-amino acids after the use of three months.

3. Results and discussion

Dynamic CSP **7** based on (R)-2-amino-1-propanol was prepared by mechanically loading (R)-N,N-carboxymethyl dodecyl-2-amino-1-propanol mono sodium salt **6** onto a commercial reversed-phase octadecyl-silica gel column as shown in Fig. 2. The diastereomeric transient ternary complex expected to be formed during the resolution of α-amino acids on dynamic CSP **7** using Cu(II) as complexing metal ion is shown in Fig. 3. The structure of the ternary complex shown in Fig. 3 is very similar to that shown in Fig. 1 except the absence of the phenyl functionality in the axial position. Therefore, based on the model shown in Figs. 1 and 3, the resolution behaviors of racemic α-amino acids on dynamic CSP **2** and **7** are expected to be quite similar except the effect of the phenyl functionality upon the chiral recognition.

Table 1 summarizes the resolution of various racemic α-amino acids on dynamic CSP **7** at constant Cu(II) concentration ($2.5 \cdot 10^{-4}$ M) with the variation of the content of organic modifier in the aqueous mobile phase. Most of the tested α-amino acids were resolved with reasonable or good separation factors. The separation factors shown in Table 1 are comparable to or better than those on dynamic CSP **2**
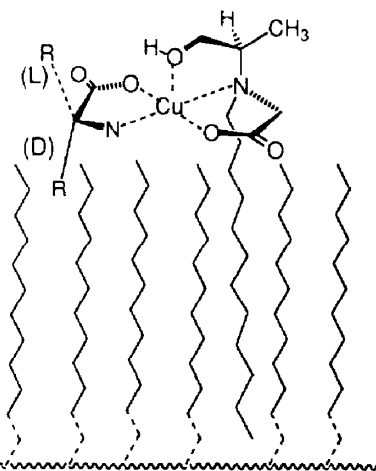

Fig. 3. The proposed structure of the ternary complex formed from the fixed ligand of CSP **7**, D- or L-amino acid and Cu(II).

Table 1
Resolution of racemic α-amino acids on dynamic CSP 7 with varying organic modifier content in the aqueous mobile phase at constant Cu(II) concentration ($2.5 \cdot 10^{-4}$ M)

Amino acid	20% MeOH		10% MeOH		100% Water		10% CH$_3$CN		20% CH$_3$CN	
	k' [a]	α [b]	k' [a]	α [b]	k' [a]	α [b]	k' [a]	α [b]	k' [a]	α [b]
Alanine	1.70 (L) 2.86 (D)	1.69	1.89 (L) 3.48 (D)	1.85	1.96 (L) 3.97 (D)	2.02	2.37 (L) 3.14 (D)	1.32	1.08 1.08	1.00
Valine	3.44 (L) 16.03 (D)	4.66	4.60 (L) 23.60 (D)	5.14			3.99 (L) 13.53 (D)	3.39	1.46 (L) 2.29 (D)	1.56
Leucine	8.33 (L) 30.89 (D)	3.71					10.14 (L) 28.74 (D)	2.83	2.25 (L) 3.36 (D)	1.52
Proline	3.86 (L) 11.41 (D)	2.95	4.76 (L) 20.50 (D)	4.30	5.49 (L) 32.14 (D)	5.85	4.24 (L) 9.45 (D)	2.23	1.37 (L) 1.75 (D)	1.28
Aspartic acid	1.18 (L) 1.52 (D)	1.28	1.92 (L) 2.69 (D)	1.40	3.54 (L) 5.21 (D)	1.47	1.28 (L) 1.51 (D)	1.18	0.03 0.03	1.00
Glutamic acid	2.47 (L) 5.10 (D)	2.06	4.04 (L) 9.54 (D)	2.36	8.27 (L) 22.07 (D)	2.67	2.57 (L) 4.77 (D)	1.86	0.17 0.17	1.00
Glutamine	1.67 (L) 2.38 (D)	1.42	2.21 (L) 3.63 (D)	1.64	2.83 (L) 5.52 (D)	1.95	2.23 (L) 2.73 (D)	1.22	0.89 0.89	1.00
Serine	1.20 (L) 1.71 (D)	1.42	1.48 (L) 2.15 (D)	1.45	1.78 (L) 2.64 (D)	1.49	1.93 (L) 2.26 (D)	1.17	1.02 1.02	1.00
Threonine	1.52 (L) 2.02 (D)	1.33	1.95 (L) 2.68 (D)	1.38	2.43 (L) 3.65 (D)	1.50	2.22 (L) 2.49 (D)	1.12	0.98 0.98	1.00
Methionine	5.83 (L) 13.55 (D)	2.32	9.06 (L) 22.81 (D)	2.52			7.02 (L) 13.68 (D)	1.95	1.71 (L) 2.21 (D)	1.29
Histidine	2.31 (D) 2.96 (L)	1.28	3.48 (D) 4.26 (L)	1.22	4.83 (D) 5.39 (L)	1.12	4.06 (D) 5.55 (L)	1.37	1.78 1.78	1.00
PheAla									3.25 (L) 5.25 (D)	1.61
PheGly									1.76 (L) 3.44 (D)	1.95
Tyrosine									1.32 (L) 1.82 (D)	1.37
Tryptophan									5.36 (L) 7.96 (D)	1.49

For chromatographic conditions, see Experimental section. For blanks, data could not be collected because of the long retention times (more than 3 h) of the two enantiomers on the column.

[a] Capacity factors for the first- and second-eluted enantiomers.
[b] Separation factors.

reported previously [14]. Particularly, the separation factors for α-amino acids containing a hydrophobic α-substituent are much greater on CSP 7 than on CSP 2 [14]. The retention times of the two enantiomers of several α-amino acids having a relatively large hydrophobic α-substituent were too long to be measured with certain mobile phases and so the resolution data for those are left blank in Table 1. An increase in the content of organic modifier in the aqueous mobile phase is found to diminish the retention of the two enantiomers on the column. Especially, the retention of D-enantiomers containing a hydrophobic α-substituent is diminished more significantly than that of L-enantiomers and, as a consequence, the separation factors decrease as the content of organic modifier in the aqueous mobile phase increases. The use of acetonitrile as an organic modifier decreases the retention of D-enantiomers much more drastically than the use of methanol as shown in Fig. 4 and, consequently, the separation factors decrease more drastically when acetonitrile is used as organic modifier. All of these trends are consistent with those on CSP 2 reported previously [14]. However, these trends are much more significant on CSP 7 than on CSP 2.

The elution orders shown in Table 1 are quite consistent. The D-enantiomers are retained always longer than the L-enantiomers except for

Fig. 4. Dependence of the retention (capacity factor, k') of D-proline and L-proline on the methanol and acetonitrile content in the aqueous mobile phase on CSP 7. For chromatographic conditions, see Experimental section.

the resolution of histidine. These elution orders are somewhat different from those on CSP 2. On CSP 2, the D-enantiomers of α-amino acids having a simple hydrophobic α-substituent were retained longer than the L-enantiomers except phenylalanine [14]. However, for α-amino acids having a hydrophilic α-substituent (e.g. aspartic acid, serine, threonine, histidine and tyrosine), L-enantiomers eluted last except glutamine and glutamic acid on CSP 2 [14].

The chiral recognition model shown in Fig. 1 has been utilized to rationalize the resolution trends and the elution orders on CSP 2 [14]. As shown in Fig. 1, the α-alkyl substituent of a D-amino acid was proposed to be intercalated between the octadecyl chains of silica gel while that of a L-amino acid directed into the bulk of mobile phase. In consequence, the ternary complex formed from a D-amino acid with a simple hydrophobic α-alkyl substituent is presumed to be more stable than that from a L-amino acid because of the lipophilic interaction between the hydrophobic α-alkyl substituent of a D-amino acid and the octadecyl chains of silica gel and, in consequence, the D-enantiomers are retained longer on the column. However, the lipophilic interaction between the hydrophobic α-alkyl substituent of a D-amino acid and the octadecyl chains of silica gel decreases as the content of organic modifier in the mobile phase increases and, as a result, the separation factors decrease.

The exactly same rationale can be applied for explaining the elution orders and the trends of retention times and separation factors for the resolution of α-amino acids with a simple hydrophobic α-alkyl substituent on CSP 7. However, it should be noted that, based on the chiral recognition model shown in Figs. 1 and 3, CSP 7 may form more stable and tighter ternary complex with Cu(II) and an α-amino acid having a simple hydrophobic α-alkyl substituent than CSP 2 because the hydroxy axial coordination is hindered by the phenyl functionality in the case of complexation with CSP 2. The tightness of the ternary complex may be greater with D-enantiomers than with L-enantiomers because of the lipophilic interaction between the hydrophobic α-alkyl substituent of a D-amino acid and the
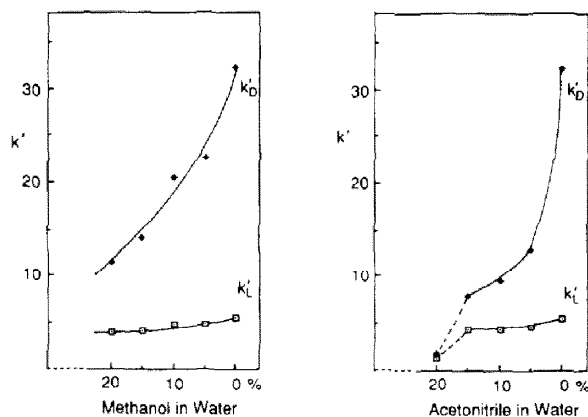
octadecyl chains of silica gel as mentioned above. In consequence, the retention times of D-enantiomers are much longer on CSP **7** than on CSP **2** for the resolution of α-amino acids having a simple hydrophobic α-alkyl substituent. The lipophilic interaction between the hydrophobic α-alkyl substituent of D-amino acids and the octadecyl chains of silica gel can be reduced quite much by the organic modifier in the aqueous mobile phase. The reduction of the lipophilic interaction between the hydrophobic α-alkyl substituent of D-amino acids and the octadecyl chains of silica gel seems to be greater with the use of less polar organic modifier in aqueous mobile phase and with tighter complexes. In this context, the use of acetonitrile as an organic modifier should decrease the retention of D-enantiomers and, as a result, the separation factors more drastically than the use of methanol. All of these trends should be more significant with tighter ternary complexes on CSP **7** than on CSP **2**. This is indeed the case as evidenced by the comparison of the resolution results on CSP **7** with those on CSP **2** [14] and by the example for the resolution trends shown in Fig. 4. Note that the high content (20%) of acetonitrile in the aqueous mobile phase reduces the retention of two enantiomers drastically as shown in Fig. 4.

In resolving the two enantiomers of α-amino acids having a hydrophilic α-alkyl substituent on CSP **2**, in our previous study, the L-enantiomers were assumed to form more stable complexes than the D-enantiomers because the extra hydrophilic functionality of the α-alkyl substituent of the L-enantiomers may interact with the hydroxy group of the fixed ligand, for example, through the hydrogen bonding or may occupy the axial coordination sphere instead of the hydroxy group of the fixed ligand [14]. As a result, the L-enantiomers are retained longer on the column than the D-enantiomers. The occupation of the axial coordination site by the hydrophilic α-alkyl substituent of L-α-amino acids during the formation of the ternary complex from the fixed ligand constituting CSP **7**, α-amino acids and Cu(II), however, seems to be more difficult and, in consequence, not to occur because the axial coordination by the hydroxy group of the fixed

ligand of CSP **7** is stronger than with CSP **2**. In this case, the stability of the ternary complex from L-enantiomers can not be greater than that from D-enantiomers. Accordingly, the elution orders on CSP **7** should be different from those on CSP **2** in resolving the two enantiomers of α-amino acids having a hydrophilic α-alkyl substituent.

However, the α-side chain of L-histidine seems to occupy the axial coordination site of the ternary complex formed with CSP **7** using the imidazole nitrogen as shown in Fig. 5a [9] possibly because of the relatively high basicity of imidazole ring [16]. It has also been reported that the α-amino group and the imidazole nitrogen of histidine may coordinate Cu(II) at the square planar coordination site to form ternary complex with another α-amino acid, with the carboxylic acid group of histidine placed in an axial position and with the two amino groups from histidine and the other amino acid in *cis*-positions of the Cu(II) coordination square [5] even though the *cis* orientation of the two amino groups is controversial [3,9]. In this context, the ternary complex formed from the fixed ligand constituting CSP **7**, L-histidine and Cu(II) has the structure shown in Fig. 5b. In both Fig. 5a and b, L-histidine is retained longer. However, it is hard to determine which mode among those shown in Fig. 5a, b and a combination of them may actually be responsible for the chiral recognition.
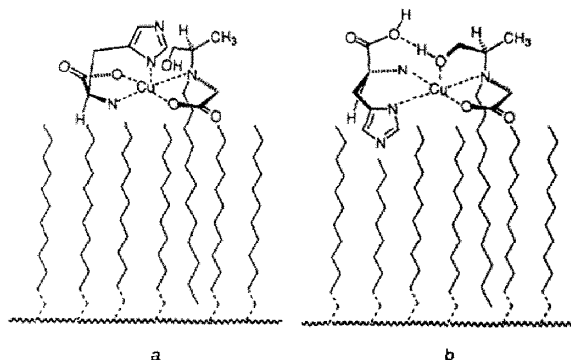

Fig. 5. The proposed structures of the ternary complex formed from the fixed ligand of CSP **7**, L-histidine and Cu(II). See text.

The variation of the Cu(II) concentration in the mobile phase of constant composition [methanol–water (20:80, v/v)] was found to affect the resolution trends for resolving the two enantiomers of α-amino acids on CSP 7 as shown in Table 2. The resolution data summarized in Table 2 indicates that the retention of the two enantiomers decreases appreciably as the Cu(II) concentration increases while the enantioselectivity denoted by the separation factors, α, does not show any noticeable trend. These tendencies are consistent with those on CSP 2 and may be

Table 2
Resolution of α-amino acids on dynamic CSP 7 with varying Cu(II) concentration in methanol–water (20:80, v/v)

Amino acid	0.10 mM Cu(II)		0.15 mM Cu(II)		0.20 mM Cu(II)		0.25 mM Cu(II)	
	k' [a]	α [b]	k' [a]	α [b]	k' [a]	α [b]	k' [a]	α [b]
Alanine	2.63 (L)	1.69	2.06 (L)	1.70	1.84 (L)	1.68	1.70 (L)	1.69
	4.44 (D)		3.52 (D)		3.10 (D)		2.86 (D)	
Valine	5.05 (L)	4.86	3.92 (L)	5.00	3.57 (L)	4.84	3.44 (L)	4.66
	24.57 (D)		19.61 (D)		17.28 (D)		16.03 (D)	
Leucine			10.08 (L)	3.93	8.88 (L)	3.86	8.33 (L)	3.71
			39.56 (D)		34.32 (D)		30.89 (D)	
Proline	6.05 (L)	2.88	4.79 (L)	2.95	4.16 (L)	3.00	3.86 (L)	2.95
	17.43 (D)		14.14 (D)		12.49 (D)		11.41 (D)	
Aspartic acid	1.52 (L)	1.27	1.55 (L)	1.28	1.42 (L)	1.30	1.18 (L)	1.28
	1.93 (D)		1.98 (D)		1.84 (D)		1.52 (D)	
Glutamic acid	3.31 (L)	2.04	3.43 (L)	2.05	3.08 (L)	2.07	2.47 (L)	2.06
	6.77 (D)		7.04 (D)		6.39 (D)		5.10 (D)	
Glutamine			1.95 (L)	1.41	1.74 (L)	1.40	1.67 (L)	1.42
			2.75 (D)		2.44 (D)		2.38 (D)	
Serine	1.85 (L)	1.41	1.56 (L)	1.42	1.30 (L)	1.42	1.20 (L)	1.42
	2.61 (D)		2.21 (D)		1.84 (D)		1.71 (D)	
Threonine	2.43 (L)	1.33	1.97 (L)	1.34	1.56 (L)	1.34	1.52 (L)	1.33
	3.23 (D)		2.65 (D)		2.09 (D)		2.02 (D)	
Methionine	9.32 (L)	2.37	7.63 (L)	2.38	6.28 (L)	2.39	5.88 (L)	2.32
	22.10 (D)		18.14 (D)		14.97 (D)		13.55 (D)	
Arginine	7.70 (L)	1.21	4.64 (L)	1.22	4.22 (L)	1.23	3.78 (L)	1.27
	9.30 (D)		5.68 (D)		5.20 (D)		4.79 (D)	
Histidine	3.88 (D)	1.27	2.78 (D)	1.33	2.59 (D)	1.28	2.31 (D)	1.28
	4.94 (L)		3.69 (L)		3.32 (L)		2.96 (L)	

For chromatographic conditions, see Experimental section. For blanks, data could not be collected because of the unreadability (glutamine at 0.10 mM $CuSO_4$) of the chromatogram or the long retention times (leucine at 0.10 mM $CuSO_4$) of the two enantiomers.

[a] Capacity factors for the first- and second-eluted enantiomers.
[b] Separation factors.

explained by the fact that the increase of the Cu(II) concentration in the mobile phase enhances the formation of the mobile binary complex from Cu(II) and resolving amino acid, and subsequently diminishes the retention of amino acids on the column as described previously in explaining the resolution trends on CSP 2 [14].

Dynamic CSP 12 based on (R)-α-phenylethylamine was prepared as shown in Fig. 2. Dynamic CSP 12 does not contain the hydroxy group which is utilized in the axial coordination shown

in Figs. 1 and 3. In consequence, it is expected that comparison of the resolution behaviors of racemic α-amino acids on dynamic CSP 12 with those on dynamic CSP 2 or 7 may explore the importance of the hydroxy axial coordination in the chiral recognition.

The resolution of various α-amino acids on dynamic CSP 12 under various conditions is summarized in Table 3. The trends shown in Table 3 in the capacity factors with the variation of the organic modifier content and the Cu(II)

Table 3
Resolution of racemic α-amino acids on dynamic CSP 12 with varying organic modifier content in the aqueous mobile phase and with varying Cu(II) concentration

Amino acid	0.10 mM CuSO₄, 10% CH₃CN		0.10 mM CuSO₄, 7% CH₃CN		0.10 mM CuSO₄, 5% CH₃CN		0.15 mM CuSO₄, 5% CH₃CN		0.20 mM CuSO₄, 5% CH₃CN	
	k' [a]	α [b]	k' [a]	α [b]	k' [a]	α [b]	k' [a]	α [b]	k' [a]	α [b]
Alanine					4.27	1.00	3.16	1.00	2.25	1.00
					4.27		3.16		2.25	
Valine	1.50	1.00	2.78 (D)	1.15	7.12 (D)	1.07	4.76 (D)	1.08	3.25 (D)	1.12
	1.50		3.20 (L)		7.64 (L)		5.15 (L)		3.64 (L)	
Leucine	2.02 (D)	1.18	4.36 (D)	1.18	9.37 (D)	1.14	6.65 (D)	1.28	4.51 (D)	1.27
	2.38 (L)		5.14 (L)		10.67 (L)		7.64 (L)		5.73 (L)	
Proline	1.18	1.00	2.53 (D)	1.15	5.65 (D)	1.11	4.29 (D)	1.12	2.82 (D)	1.14
	1.18		2.91 (L)		6.15 (L)		4.79 (L)		3.21 (L)	
Aspartic acid	0.19	1.00	0.39	1.00	1.23	1.00	1.07	1.00	0.92	1.00
	0.19		0.39		1.23		1.07		0.92	
Glutamine	1.10	1.00	2.39	1.00	4.40	1.00	3.00	1.00	2.38	1.00
	1.10		2.39		4.40		3.00		2.38	
Methionine	2.08	1.00	3.92 (D)	1.00	8.05 (D)	1.11	5.68 (D)	1.11	4.16 (D)	1.12
	2.08		5.47 (L)		8.95 (L)		6.28 (L)		4.64 (L)	
Histidine	1.95	1.00	3.63	1.00	8.23	1.00	5.74	1.00	3.60	1.00
	1.95		3.63		8.23		5.74		3.60	
PheGly	1.91 (D)	1.22	3.95 (D)	1.23	8.53 (D)	1.18	5.92 (D)	1.18	4.23 (D)	1.20
	2.33 (L)		4.85 (L)		10.04 (L)		6.99 (L)		5.19 (L)	
Tyrosine	2.15	1.00	3.94 (D)	1.09	9.23	1.00	6.52	1.00	5.22	1.00
	2.15		4.30 (L)		9.23		6.52		5.22	

For chromatographic conditions, see Experimental section. For blanks, data were not collected.
[a] Capacity factors for the first and second-eluted enantiomers.
[b] Separation factors.

concentration in the aqueous mobile phase are consistent with those on CSP 2 or CSP 7 and can be rationalized as described above or previously [14]. However, the degree of enantioselectivity denoted by the separation factors, α, on CSP 12 is much worse than that on CSPs 2 or 7. The elution orders on CSP 12 are totally different from those on CSPs 2 or 7. These results indicate that the axial coordination by the hydroxy group shown in Figs. 1 and 3 is very important in the chiral recognition.

The chiral recognition model for resolving α-amino acids on CSP 12 is proposed as shown in Fig. 6. To minimize the disturbance of steric hindrance in forming the ternary complex, the least bulky hydrogen at the chiral center of the fixed ligand is assumed to be positioned above the metal ion in Fig. 6. In this instance, the phenyl and methyl groups at the chiral center of the fixed ligand are placed as shown in Fig. 6a or b. The complex shown in Fig. 6a is quite similar to that shown in Figs. 1 and 3 except the absence of the hydroxy axial coordination and the D-enantiomers are selectively retained because of the lipophilic interaction between the side α-alkyl chains of D-enantiomers and the octadecyl
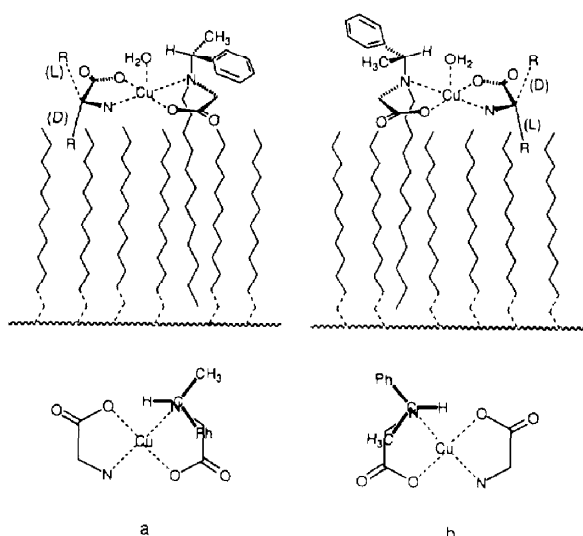

Fig. 6. Top: the proposed structures of the ternary complex formed from the fixed ligand of CSP 12, D- or L-amino acid and Cu(II). Bottom: schematic representation of the top ternary complexes viewed from above. See text.

chains of silica gel as noted in Figs. 1 and 3. In Fig. 6a, however, the most bulky phenyl group is eclipsed with the N-carboxymethyl unit of the fixed ligand and some steric hindrance is expected. To lessen the steric hindrance between the phenyl group and the N-carboxymethyl unit of the fixed ligand during the formation of the ternary complex, the bonding direction of the N-carboxymethyl unit of the fixed ligand is reversed as shown in Fig. 6b and, in consequence, the phenyl group is positioned out of the N-carboxymethyl sphere of the fixed ligand. On this occasion, the L-enantiomers are selectively retained because of the lipophilic interaction between the side α-alkyl chains of L-enantiomers and the octadecyl chains of silica gel. Based on the structure of the two complexes shown in Fig. 6, it is concluded that the complex which selectively retains the L-enantiomers is more stable than that which selectively retains the D-enantiomers. Consequently, the L-enantiomers are retained longer on dynamic CSP 12 than the D-enantiomers. However, the stability difference between the two complexes shown in Fig. 5 is presumed to be quite small based on the degree of enantioselectivity shown in Table 3.

In summary, in the present study, we prepared dynamic CSP 7 from (R)-2-amino-1-propanol and dynamic CSP 12 from (R)-α-phenylethylamine and resolved the two enantiomers of various α-amino acids on the two new CSPs. CSP 7 does not contain any phenyl functionality which is present at the first chiral center of CSP 2 and CSP 12 does not contain any hydroxy functional group which occupies the axial coordination site in the ternary complex formed from CSP 2 or 7. Based on the trends for resolving α-amino acids on CSP 7 and the comparison of these trends with those on CSP 2, we concluded that the phenyl functionality at the first chiral center of CSP 2 is not essential in the chiral recognition and simply disturbs the axial coordination of the hydroxy group of the fixed ligand in the square planar coordination sphere of the ternary complex. In consequence, CSP 7 with its lack of the phenyl functionality forms a more stable and tighter complex and, as a result, shows higher enantioselectivity for the two en-

antiomers of α-amino acids having a simple hydrophobic α-alkyl substituent than CSP **2** and different elution orders from CSP **2** in resolving α-amino acids having a hydrophilic α-alkyl substituent. On the other hand, the enantioselectivity for the two enantiomers of α-amino acids on CSP **12** was much worse than that on CSPs **2** or **7** and the elution orders were totally different from those on CSPs **2** or **7**. From these results, the hydroxy group of CSPs **2** and **7** is concluded to play a very important role in the chiral recognition.

Acknowledgement

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