Comparison of the chiral resolution on *cis–trans* isomeric chiral stationary phases derived from (S)-1-(1-naphthyl)ethylamine

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Abstract

A pair of *cis–trans* isomeric chiral stationary phases (CSPs) derived from (S)-1-(1-naphthyl)ethylamine was prepared. The chromatographic behaviours on both CSPs with regard to the resolution of enantiomeric amino acids, amino alcohols, amines, and carboxylic acid were studied. According to separation factors, the *trans*-CSP showed better chiral recognition ability for the separation of most analytes chosen in this study. Three homologous series of the alkyl esters of racemic amino acids were resolved on both CSPs using *n*-hexane–2-propanol and *n*-hexane–dichloromethane as mobile phases. The *trans*-CSP also showed better enantioselectivity for the resolution of homologues. A reverse of elution order was observed for the resolution of the homologous series of phenylglycine alkyl esters on both CSPs. It was found that the relationship between the separation factor and the alkyl chain length of the ester homologous series depended upon the components of mobile phase. A higher magnitude of difference between the two CSPs in enantioselectivity for the resolution of a given homologue was obtained when *n*-hexane–dichloromethane was used as a mobile phase. A chiral recognition process, in which steric repulsion, face-to-face $\pi-\pi$ interaction, face-to-edge $\pi-\pi$ interaction and hydrogen bonding interaction were involved, was also suggested to describe the separation of enantiomeric homologues on both CSPs. This study clearly indicates that the chiral resolution is influenced by the geometry of the double bond in a CSP.

Keywords: Chiral stationary phases, LC; Enantiomer separation; Naphthylethylamine; Amino acids; Amino alcohols; Amines; Ibuprofen

1. Introduction

Several chiral stationary phases (CSPs) derived from 1-arylalkylamine derivatives showed effective enantiomeric separation of various analytes by high-performance liquid chromatography [1–8]. According to the connecting site of chiral moiety in which the chiral moiety was connected to silica gel surface,

three typical types of CSPs (CSPs A–C, Fig. 1) have been prepared [1–4]. The connecting site of chiral moiety was acyl group in CSP A, whereas those were the alkyl and naphthyl groups in CSP B and CSP C, respectively.

The resolution of homologous series of enantiomeric analytes on these CSPs has been found to be dependent on the connecting site of chiral moiety [2,10,11]. For example, as the alkyl chain length of the homologous analytes of 1-arylalkylamines increased, the enantioselectivity decreased on CSP A
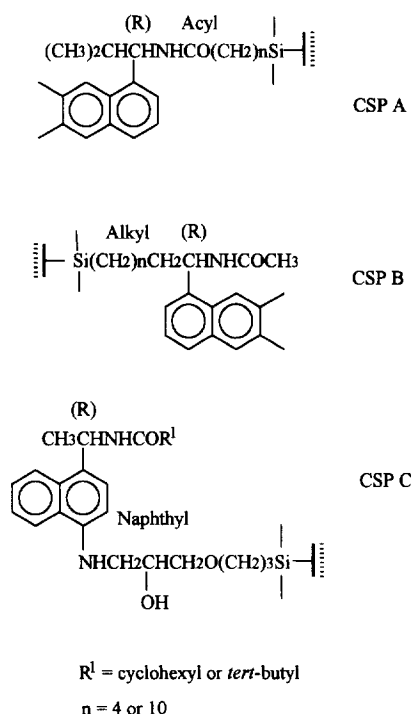
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(R)   Acyl

(CH3)2CHCHCHNHCO(CH2)nSi—

CSP A

Alkyl   (R)

Si(CH2)nCH2CHNHCOCH3

CSP B

(R)
CH3CHNHCOR¹

CSP C

Naphthyl

NHCH2CHCH2O(CH2)3Si—
   OH

R¹ = cyclohexyl or *tert*-butyl

n = 4 or 10

Fig. 1. Structures of CSPs A–C.

(S)
CH3CHNHC—CONHCH2CH2CH2Si—   CSP 1

(S)
CH3CHNHC—CONHCH2CH2CH2Si—   CSP 2

Fig. 2. Structures of CSP 1 and CSP 2.

and increased on CSPs B and C. The chiral recognition mechanism on the 1-arylalkylamine-derived CSPs was also proposed [9]. Steric repulsive interaction, hydrogen bonding, face-to-face π–π interaction and face-to-edge π–π interaction were involved in the chiral recognition. The different orientation of the chiral selector with respect to the silica gel surface resulted in different chiral recognition behaviours among these three types of CSPs.

It has been found that the *R,S* absolute configuration of a chiral selector affected the chiral resolution. For example, opposite configurations of the last eluted enantiomers were always observed for the resolution of a given analyte on a pair of enantiomeric CSPs, and enantioselectivity was usually influenced by adding an additional chiral centre to a chiral selector [7,12–14]. However, the influence of the *cis–trans* configuration of a CSP on chiral discrimination is still not clear. Due to the different spatial orientation of a pair of *cis–trans* geometry isomers, it seems that chiral resolution of homologous enantiomers should also be affected by the *cis–trans* configuration of the chiral selector. Several recently reported CSPs contained either *cis*- or *trans*-
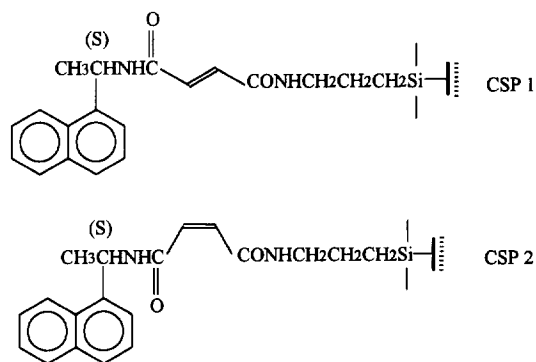
chiral selector [15–18]. The increasing development in the *cis*- and *trans*-CSPs also aroused our interest in comparing the chromatographic behaviours on a pair of *cis–trans* isomeric CSPs derived from the same chiral entity.

This study demonstrates the influence of the *cis–trans* configuration of a CSP on the chiral resolution of enantiomers. As shown in Fig. 2, both CSPs have the same connecting site of the chiral moiety, 1-naphthylethylamine. However, the configuration of the double bond in CSP 1 is *trans*- and that in CSP 2 is *cis*-configuration. Chromatographic results showed that the *trans*-CSP has better chiral recognition ability than the *cis*-CSP for the resolution of most of the chosen analytes. The enantioselectivity for the resolution of homologues on *trans*-CSP was also better than that on *cis*-CSP, and the magnitude of difference between the two CSPs in enantioselectivity depended on the components of mobile phase. Using *n*-hexane–dichloromethane as a mobile phase, a higher magnitude of difference between the two CSPs in enantioselectivity was observed. The present study shows that the chiral resolution is influenced by the *cis–trans* configuration of a chiral selector.

2. Experimental

2.1. Chemicals

The silica gel (Nucleosil; pore size 100 Å, particle size 5 μm) was obtained from Macherey-Nagel. 3-Aminopropyltriethoxysilane (APS) was purchased from Chisso. Maleic anhydride was of reagent grade and supplied from Wako (Japan). Fumaric acid was

obtained from Merck. (S)-1-(1-Naphthyl)ethylamine and (2-ethoxy-1-ethxoycarbonyl-1,2-dihydroquinoline (EEDQ) were from Aldrich. The analytes used in the chromatographic experiments were of synthetic reagent grade and obtained from Aldrich or Merck.

2.2. Preparation of APS-modified silica gel

The procedure for the preparation of APS-modified silica gel was the same as that reported previously [12]. Analysis: found, C 3.72, H 1.07, N 1.30%. Calculated: 0.93 mmol of ligand/g stationary phase (based on N).

2.3. Preparation of (S)-N-(1-naphthylethyl)fumaric acid monoamide

Fumaric acid (1.4 g, 0.012 mol) and EEDQ (3.0 g, 0.012 mol) were dissolved in 30 ml of tetrahydrofuran (THF) under nitrogen, and 3.0 g (0.03 mol) of triethylamine was added. A solution of (S)-1-(1-naphthyl)ethylamine (1.7 g, 0.01 mol) in THF (10 ml) was slowly dripped into the reaction mixture. The mixture was stirred for 3 h at room temperature, and then poured into 200 ml of 0.1 M aqueous NaOH. After removal of the suspended solid, the clear aqueous solution was acidified with dilute hydrochloric acid and a crude product was precipitated as a white solid. The resulting white solid was collected by filtration, washed with 50 ml of water and dried under vacuum in the presence of P_2O_5. After recrystallization from methanol, 1.1 g (42%) of the product was obtained; m.p. 230–232°C; ^{1}H NMR ([$^2H_6$]dimethyl sulfoxide (DMSO-d_6), 200 MHz) δ 1.53 (d, 3H, CH3), 5.78 (m, 1H, CH), 6.53 (d, $J=15$ Hz, 1H, C=CH), 7.02 (d, J=15 Hz, 1H, C=CH), 7.45–8.12 (m, 7H, ArH), 9.11 (d, 1H, NH), 12.91 (bs, 1H, COOH); IR (KBr) 3300, 2500–3000, 3010, 2970, 1700, 1650. Analysis: calculated for $C_{16}H_{15}NO_3$, C 71.38, H 5.57, N 5.20%; found, C 71.00, H 5.52, N 5.41%.

2.4. Preparation of (S)-N-(1-naphthylethyl)maleic acid monoamide

Sodium bicarbonate (1.7 g, 0.02 mol) was dissolved in water (100 ml) and (S)-1-(1-naphthyl)ethylamine (1.7 g, 0.01 mol) was added. The mixture was stirred vigorously under nitrogen, and 0.98 g of maleic anhydride (0.01 mol) was added. After stirring for 1 h, the reaction solution was acidified with dilute hydrochloric acid, and extracted with 2-portion of 50 ml diethyl ether. The ether layer was dried by anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to give the crude product as a white solid. After recrystallization from methanol, 2.3 g (87%) of product was obtained; m.p. 135–136°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ 1.55 (d, 3H, CH3), 5.78 (m, 1H, CH) 6.26 (d, $J=12$ Hz, 1H, C=CH), 6.44 (d, $J=12$ Hz, 1H, C=CH), 7.52–8.13 (m, 7H, ArH), 9.51 (d, 1H, NH), 12.91 (bs, 1H, COOH); IR (KBr) 2800–3400, 3240, 3010, 2970, 1700, 1630. Analysis: calculated for $C_{16}H_{15}NO_3$, C 71.38, H 5.57, N 5.20%; found, C 71.07, H 5.53, N 5.39%.

2.5. Preparation of (R)-N-(1-naphthylethyl) phthalamic acid

Sodium hydroxide (0.8 g, 0.02 mol) was dissolved in water (100 ml) and (R)-1-(1-naphthyl)ethylamine (1.7 g, 0.01 mol) was added. The mixture was stirred vigorously under nitrogen, and 1.48 g of phthalic anhydride (0.01 mol) was added. After stirring for 1 h, the reaction solution was acidified with dilute hydrochloric acid. The resulting white solid was collected by filtration, washed with water, and dried under vacuum in the presence of P_2O_5. After recrystallization from methanol, 2.71 g (85%) of product was obtained; m.p. 153–154°C; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 1.57 (d, 3H, CH3), 5.87 (m, 1H, CH), 7.35–8.23 (m, 11H, ArH), 8.91 (d, 1H, NH), 9.52 (bs, 1H, COOH); IR (KBr) 3270, 2600–3300, 3050, 2970, 1700, 1640. Analysis: calculated for $C_{20}H_{17}NO_3$, C 75.24, H 5.33, N 4.39%; found, C 74.63, H 5.45, N 4.40%.

2.6. Preparation of CSPs 1 and 2 and CSPs D and E

(S)-N-(1-Naphthylethyl)fumaric acid monoamide (1.4 g, 5 mmol) and EEDQ (1.2 g, 5 mmol) were dissolved in THF (100 ml), and 2.5 g of APS-silica gel was added. The mixture was subjected to ultrasonic vibration for 10 min and then stirred gently for 48 h. The prepared CSP 1 was collected by filtration and washed thoroughly with THF, water,

methanol and ether, and dried under vacuum in the presence of P_2O_5. Analysis found: C 15.13, H 2.22, N 2.05%. Calculated: 0.53 mmol of (S)-ligand/g stationary phase (based on N).

Analogously to CSP 1, CSP 2 was obtained from (S)-N-(1-naphthylethyl)maleic acid monoamide. Analysis found: C 13.82, H 2.05, N 2.16%. Calculated: 0.59 mmol of (S)-ligand/g stationary phase (based on N).

Analogous to CSP 1, CSP D was obtained from (S)-N-(1-naphthylethyl)succinamic acid which was prepared according to the procedure described by Oi et al. [5]. Analysis found: C 17.50, H 2.35, N 2.46%. Calculated: 0.87 mmol of (S)-ligand/g stationary phase (based on N).

Analogous to CSP 1, CSP E was obtained from (R)-N-(1-naphthylethyl)phthalamic acid and end-capped by reaction with trimethylsilane. Analysis found: C 11.29, H 2.12, N 1.83%. Calculated: 0.38 mmol of (R)-ligand/g stationary phase (based on N).

2.7. Chromatographic studies

The chromatographic studies were carried out with a liquid chromatographic system consisting of an Alphatech solvent-delivery system and an Applied Biosystems Model 757 variable-wavelength UV detector. The recorder used was a Model 21 SIC Chromatocorder. Stainless-steel columns (250×4 mm I.D.) were packed by the balance-density slurry method using an Econo-packing pump (Inpac International) at 600 kg/cm². Binary solvents of *n*-hexane–2-propanol (50:50–80:20) and *n*-hexane–dichloromethane (45:55–70:30) were used as the mobile phase, which was filtered and degassed by ultrasonic vibration prior to use. The flow-rate was 1.0 ml/min. The detector was operated at 254 nm. Experiments were carried out at 25°C. Chromatographic peaks were assigned by injecting the corresponding derivative of the enantiomerically enriched analyte.

3. Results and discussion

CSP 1 and CSP 2 were prepared by bonding chiral monoamides to APS-modified silica gel. (S)-N-(1-Naphthylethyl)maleic acid monoamide was synthes-

ized by the reaction of maleic anhydride with 1-naphthylethylamine in aqueous solution. Whereas, (S)-N-(1-naphthylethyl)fumaric acid monoamide was prepared by the reaction of fumaric acid and 1-naphthylethylamine in THF using EEDQ as dehydration agent. Before recrystallization, the removal of diamide and unreacted fumaric acid was necessary for the preparation of the latter monoamide. In order to compare the chromatographic behaviours on CSPs 1 and 2, the preparation and packing procedure of both CSPs were carried out under identical conditions. According to the elementary analyses, the surface coverage of chiral ligands on CSP 1 and CSP 2 were similar.

3.1. Chromatographic behaviours of the prepared CSPs

Chromatographic results for the resolution of twelve enantiomeric analytes on CSPs 1 and 2 were examined (Table 1). According to the separation factors, the chiral recognition ability of CSP 1 was better than that of CSP 2 for the resolution of most of the enantiomeric analytes chosen in this study. The separation factor of a given analyte, except leucine and phenylalanine, on CSP 1 was higher than that on CSP 2. The chromatographic results on CSPs 1 and 2 were also compared with those on CSPs A–C that have been reported in literature [2,4,8,10,11]. Some instances on CSPs A–C were better whereas some instances on CSPs 1 and 2 were better.

3.2. Elution orders of enantiomeric amino acids

For the resolution of phenylethylamine and naphthylethylamine analytes, the elution orders on CSPs 1 and 2 and CSPs A–C were the same. The absolute configurations of the chiral selectors in CSPs 1 and 2 were (S)- and those in CSPs A–C were (R)-configuration. The last eluted enantiomers of analytes had the same absolute configuration with chiral selectors in CSPs 1 and 2 and CSPs A–C. For example, the (R)-phenylethylamine, as 3,5-dinitrobenzamide derivative, was the last eluted enantiomer on (R)-CSP A and the (S)-phenylethylamine was the last eluted enantiomer on (S)-CSP 1.

However, the elution orders of amino acid ana-

Table 1
Resolution of derivatized enantiomers on CSPs 1 and 2

Analyte	CSP1				CSP2			
	k_1'	α	Conf.	M	k_1'	α	Conf.	M
Amino acids[a]								
Valine	5.34	2.18	S	B	9.57	2.01	S	C
Alanine	4.35	1.84	S	C	6.75	1.67	S	B
Leucine	2.79	1.81	S	C	2.63	1.85	S	C
Methionine	6.65	1.95	S	C	10.5	1.80	S	B
Phenylalanine	8.26	1.40	S	C	12.0	1.45	S	D
Phenylglycine	11.8	1.04	R	B	31.1	1.04	R	B
Amino alcohols[b]								
2-Aminobutanol	2.83	1.21		C	5.24	1.18		B
2-Aminopropanol	4.08	1.13	S	C	7.47	1.11	S	B
Norephedrine	4.06	1.19		C	7.81	1.14		B
Amines[b]								
Phenylethylamine	10.2	2.36	S	B	9.37	1.96	S	B
Naphthylethylamine	12.7	4.31	S	B	11.6	2.98	S	B
Carboxylic acid[c]								
Ibuprofen	3.77	3.11	R	B	4.51	2.20	R	B

k_1' is the capacity factor of the first eluted enantiomer; Conf. indicates the absolute configuration of the last-eluted enantiomer. The separation factor (α) is the ratio of the capacity factors of enantiomers. Mobile phase (M): A=n-hexane–2-propanol (90:10); B=n-hexane–2-propanol (80:20); C=n-hexane–2-propanol (70:30); D=n-hexane–2-propanol (60:40).
[a] As N-3,5-dinitrobenzamide-O-methyl ester derivatives.
[b] As N-3,5-dinitrobenzamide derivatives.
[c] As 3,5-dinitroanilide derivative.

lytes on CSPs 1 and 2 and CSPs A–C were interesting. For the resolution of phenylglycine methyl ester, the elution order on CSPs 1 and 2 was opposite to that on CSPs A–C. On the other hand, for the resolution of the other amino acids chosen in this study, the elution orders were the same on both CSPs 1 and 2 and CSPs A–C. The absolute configuration of the last eluted enantiomer of phenylglycine methyl ester, as 3,5-dinitrobenzamide, was opposite to the configuration of CSPs 1 and 2 and identical to the configuration of CSPs A–C. The (R)-phenylglycine was the last eluted enantiomer on both the (S)-CSPs 1 and 2 and the (R)-CSPs A–C. For the resolution of the other amino acid analytes chosen in this study, the absolute configurations of the last eluted enantiomers were the same as those of CSPs 1 and 2 and CSPs A–C. The (S)-enantiomers were the last eluted enantiomers on (S)-CSPs 1 and 2 and the (R)-enantiomers were the last eluted enantiomers on (R)-CSPs A–C.

The elution orders of amino acid analytes on CSPs 1 and 2 were further compared with those on CSPs D

and E (Fig. 3), the structures of which are similar to those of CSPs 1 and 2. CSP D has been prepared and the data of the elution orders of all the analytes chosen in this study, except phenylglycine, on CSP D were obtained from Ref. [5], whereas the data of the elution order for the resolution of phenylglycine methyl ester on CSP D was obtained from the CSP
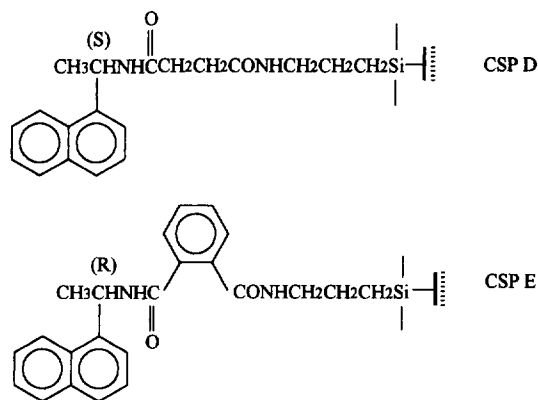

Fig. 3. Structures of CSP D and CSP E.

prepared in this study. The same elution orders were observed on CSPs 1 and 2 and CSPs D and E for the resolution of the chosen enantiomeric amino acids.

3.3. Resolution of homologous series of analytes on CSPs 1 and 2 using n-hexane–2-propanol as a mobile phase

In order to obtain more information of chiral recognition mechanism on CSPs 1 and 2, three analytes, valine, phenylalanine and phenylglycine, were derivatized with 3,5-dinitrobenzoyl chloride and esterified with various alcohols (Fig. 4). Fig. 5 shows the relationship of the separation factor and the alkoxyl chain length of analytes for the enantiomeric separation of homologues on CSPs 1 and 2 using n-hexane–2-propanol as a mobile phase. The (S)-enantiomers were always the last eluted enantiomers for the resolution of the homologous analytes derived from valine and phenylalanine. However, a reverse of elution order was observed for the resolution of homologues derived from phenylglycine. The (R)-enantiomer was the last eluted enantiomer for the resolution of phenylglycine methyl ester, on the other hand, the (S)-enantiomers were last eluted for the resolution of the other homologues with n higher than 2. The elution orders of each homologue were consistent under the investigated compositions of mobile phase (Table 2).

Both CSP 1 and CSP 2 showed a similar trend of

relationship between the separation factor and the alkoxyl chain length for the resolution of each homologous series (Fig. 5). For the resolution of the homologous series derived from valine and phenylalanine, an increase in separation factor was found as the alkoxyl chain length increased. For the resolution of homologues derived from phenylglycine, an initial decrease, and then an increase in separation factor was found as the value of the n in homologues increased. The relationship between separation factor and alkoxyl chain length of each homologous series was also consistent under various investigated compositions of the n-hexane–2-propanol mobile phase (Table 2).

However, the separation factor of each enantiomeric homologue on trans-CSP 1 was higher than that on cis-CSP 2 (Fig. 5). For example, each homologue of analyte 1 had higher separation factor on CSP 1. For the resolution of the homologues derived from phenylalanine, the methyl ester showed no significant difference in separation factors on both CSPs, however, the other esters of phenylalanine had higher separation factors on CSP 1.

The magnitude of difference between the two CSPs in separation factors of each homologue increased as the chain length of alkoxyl group increased (Fig. 5). For example, there was no significant difference in separation factors for the resolution of the methyl ester of phenylalanine on both CSPs, however, the magnitude of difference in the α
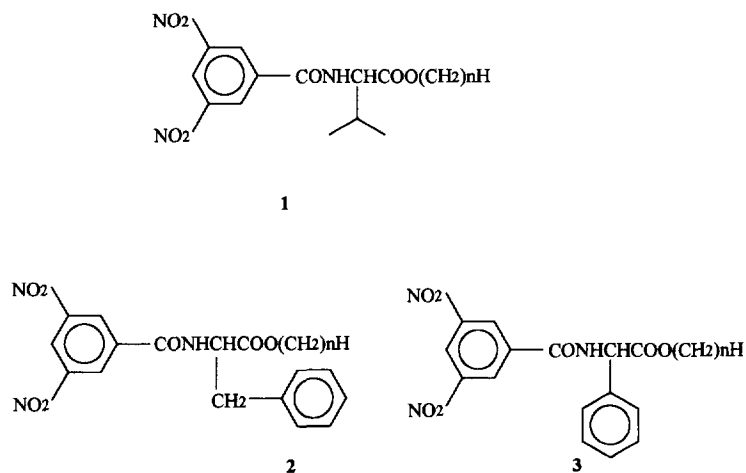

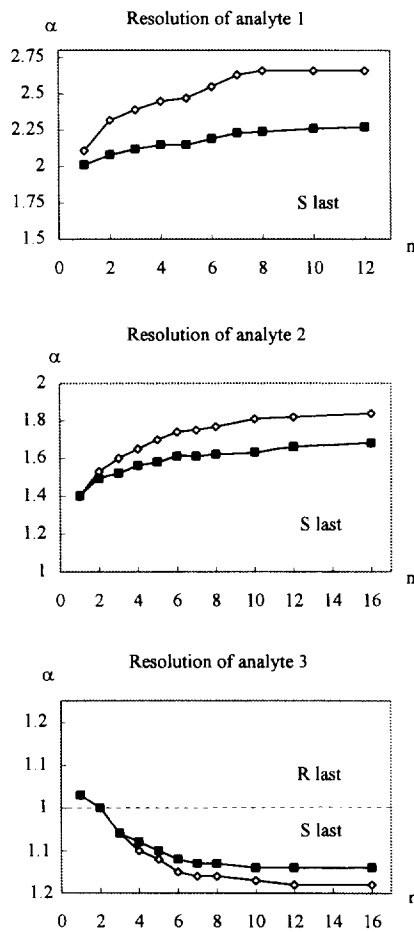Fig. 4. Structures of the homologous series of analytes.

Fig. 5. Separation of enantiomeric homologues on CSPs 1 and 2 using n-hexane–2-propanol (70:30) as a mobile phase. ◇=CSP 1; ■=CSP 2.

graphic behaviours were observed on both CSPs. As the alkoxyl chain length increased, an increase in separation factor was found on CSP 1, however, a decrease in separation factor was found on CSP 2. For the resolution of analyte 2, the separation factors on CSP 2 almost remained constant whereas the separation factors on CSP 1 increased as the alkoxyl chain length increased. For the resolution of analytes 3, a reverse of elution order was also observed on CSP 1 (Fig. 6). However, only homologous analyte 3 with shorter alkoxyl chain could be resolved on CSP
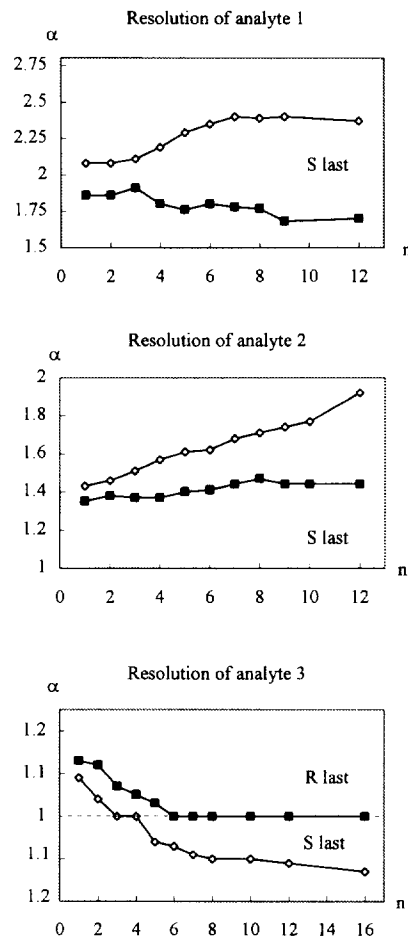
Fig. 6. Chiral resolution of enantiomeric homologues on CSPs 1 and 2 using n-hexane–dichloromethane as a mobile phase. Compositions of mobile phase: n-hexane–dichloromethane (70:30) for the resolution of analyte 1, n-hexane–dichloromethane (60:40) for the resolution of analyte 2 and analyte 3; ◇=CSP 1; ■=CSP 2.

values of homologue on both CSPs were increased as the chain length of alkoxyl group increased.

3.4. Resolution of homologous series of analytes on CSPs 1 and 2 using n-hexane–dichloromethane as a mobile phase

Using n-hexane–dichloromethane as a mobile phase, the relationships of the separation factor and alkoxyl chain length are demonstrated in Fig. 6 and Table 2. For the separation of homologous series derived from valine, dramatically different chromato-

Table 2
Chiral resolution of homologues on CSPs 1 and 2

Analyte	CSP1								CSP2							
	n-Hexane–2-propanol[a]				n-Hexane–dichloromethane[a]				n-Hexane–2-propanol[a]				n-Hexane–dichloromethane[a]			
	60:40		80:20		50:50		70:30		60:40		80:20		50:50		70:30	
n	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α	k'_1	α
Analyte 1																
1	2.09	2.17(S)	5.34	2.18(S)	0.96	2.0(S)	4.96	2.08(S)	2.00	2.00(S)	4.55	2.00(S)	1.86	1.68(S)	7.70	1.86(S)
3	1.41	2.40(S)	3.24	2.44(S)	0.55	2.2(S)	2.51	2.11(S)	1.48	2.11(S)	3.07	2.12(S)	1.04	1.92(S)	4.26	1.91(S)
5	1.17	2.47(S)	2.62	2.51(S)	0.44	2.2(S)	2.09	2.29(S)	1.30	2.16(S)	2.67	2.15(S)	1.03	1.90(S)	4.24	1.76(S)
8	1.04	2.61(S)	2.29	2.66(S)	0.34	2.6(S)	1.61	2.39(S)	1.16	2.23(S)	2.32	2.24(S)	0.82	1.8(S)	3.22	1.77(S)
12	0.88	2.7(S)	1.98	2.37(S)	0.27	2.7(S)	1.45	2.37(S)	1.01	2.28(S)	2.04	2.26(S)	0.64	1.8(S)	2.52	1.70(S)
Analyte 2																
1	5.45	1.40(S)	13.8	1.38(S)	2.15	1.30(S)	12.5	1.32(S)	4.62	1.45(S)	12.0	1.42(S)	4.14	1.36(S)	20.7	1.25(S)
3	3.36	1.60(S)	7.60	1.60(S)	1.32	1.52(S)	6.24	1.52(S)	3.52	1.53(S)	7.50	1.53(S)	3.09	1.35(S)	12.2	1.35(S)
5	2.74	1.70(S)	6.11	1.73(S)	1.01	1.63(S)	4.79	1.67(S)	3.08	1.58(S)	6.26	1.60(S)	2.54	1.40(S)	9.29	1.42(S)
8	2.32	1.78(S)	5.12	1.81(S)	0.80	1.7(S)	3.93	1.70(S)	2.67	1.62(S)	5.31	1.64(S)	1.85	1.50(S)	7.79	1.53(S)
12	2.06	1.82(S)	4.49	1.85(S)	0.64	1.8(S)	3.05	1.95(S)	2.14	1.65(S)	4.78	1.66(S)	1.44	1.61(S)	6.08	1.58(S)
Analyte 3																
1	7.46	1.03(R)	19.8	1.04(R)	2.39	1.14(R)	17.5	1.10(R)	5.82	1.00	13.8	1.04(R)	4.53	1.12(R)	22.1	1.15(R)
2	5.86	1.00	13.3	1.00	2.08	1.04(R)	10.6	1.05(R)	4.57	1.00	10.6	1.00	3.33	1.08(R)	15.6	1.12(R)
3	4.85	1.06(S)	10.5	1.08(S)	1.67	1.00	8.92	1.00	3.98	1.05(S)	8.51	1.06(S)	2.59	1.00	13.0	1.07(R)
5	4.10	1.12(S)	8.74	1.15(S)	1.45	1.03(S)	6.66	1.07(S)	3.40	1.09(S)	8.26	1.11(S)	2.48	1.00	11.8	1.00
8	3.41	1.16(S)	7.13	1.19(S)	1.11	1.08(S)	5.30	1.11(S)	2.93	1.12(S)	6.80	1.15(S)	2.08	1.00	9.81	1.00
12	3.00	1.17(S)	7.27	1.19(S)	0.87	1.1(S)	3.24	1.14(S)	2.61	1.13(S)	6.04	1.16(S)	1.53	1.00	7.93	1.00

k'_1 is the capacity factor of the first eluted enantiomer. The separation factor (α) is the ratio of the capacity factors of enantiomers; α(R/S) indicates the absolute configuration of the last-eluted enantiomer.

[a] Mobile phase used.

2, no significant resolution was observed on CSP 2 for the resolution of the other homologues. Using *n*-hexane–dichloromethane as a mobile phase, a larger magnitude of difference between CSP 1 and CSP 2 in enantioselectivity was found for the resolution of the three homologous series.

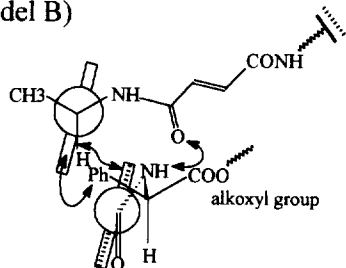## 3.5. Chiral recognition process on CSPs 1 and 2

To rationalize the observed chromatographic behaviours, chiral recognition models were proposed. The chiral recognition models for the resolution of the homologous analytes 1–3 on CSP 1 are demonstrated in models A and B (Fig. 7). Model A shows the more stable homochiral (S)-(S) complex for the resolution of homologues derived from valine or phenylalanine on CSP 1. The face-to-face π–π interaction and hydrogen bonding are involved in the

recognition process. The alkoxyl group does not direct toward the silica surface, thus non-intercalative chromatographic results were found. However, for the resolution of homologues derived from phenylglycine on CSP 1, in addition to the face-to-face π–π interaction and hydrogen bonding interaction, a face-to-edge π–π interaction is also involved in chiral discrimination process. Owing to the additional face-to-edge π–π interaction, the heterochiral (R)–(S) complex (model B, Fig. 7) becomes more stable, instead of the corresponding homochiral complex, for the resolution of phenylglycine methyl ester on CSP 1. It should be noted that the alkoxyl group of a homologue derived from phenylglycine is parallel to the strains of CSP 1 in the heterochiral complex. As the chain length of the alkoxyl group increases, the corresponding homochiral complex becomes more stable because of intercalative effect.

(Model A)
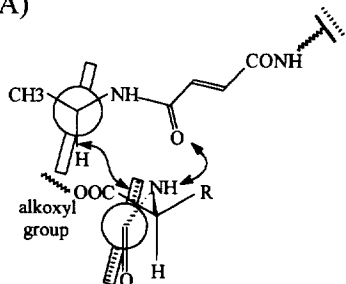
(Model B)

R = isopropyl group,   Ph = phenyl group
benzyl group

▢ = naphthyl group   ▢ = 3,5-dinitrophenyl group

Fig. 7. Schematic representation of the proposed chiral recognition models on CSP 1. Model A: homochiral complex with (S)-enantiomer of analyte 1 or analyte 2; model B: heterochiral complex with (R)-enantiomer of analyte 3.

Table 3
Chiral resolution of homologues on (S)-CSP D and (R)-CSP E

Analyte	(S)-CSP D				(R)-CSP E			
n	k'_1	α	Conf.	M	k'_1	α	Conf.	M
Analyte 1								
1	2.15	2.68	S	C	2.56	1.73	R	A
3	1.44	3.11	S	C	1.60	1.67	R	A
5	1.09	3.28	S	C	1.36	1.65	R	A
8	0.95	3.5	S	C	1.20	1.61	R	A
12	0.83	3.7	S	C	1.20	1.58	R	A
Analyte 2								
1	6.05	1.67	S	C	4.34	1.24	R	B
3	3.41	2.12	S	C	2.61	1.21	R	B
5	2.68	2.41	S	C	2.17	1.19	R	B
8	2.24	2.57	S	C	1.90	1.18	R	B
12	1.97	2.61	S	C	1.68	1.16	R	B
Analyte 3								
1	5.22	1.12	R	D	3.32	1.14	S	B
2	3.45	1.00		D	2.32	1.12	S	B
3	2.63	1.00		D	2.00	1.09	S	B
5	1.99	1.11	S	D	1.75	1.03	S	B
8	1.48	1.20	S	D	1.48	1.00		B
12	1.16	1.23	S	D	1.33	1.00		B

k'_1 is the capacity factor of the first eluted enantiomer; Conf. indicates the absolute configuration of the last-eluted enantiomer. The separation factor (α) is the ratio of the capacity factors of enantiomers. Mobile phase (M): A=*n*-hexane–2-propanol (80:20); B=*n*-hexane–2-propanol (70:30); C=*n*-hexane–2-propanol (60:40); D=*n*-hexane–dichloromethane (50:50).

Thus, the reverse of elution order occurred in the resolution of the homologues derived from phenylglycine on CSP 1 could be illustrated.

Unlike CSP 1, CSP 2 is conformationally heterogeneous. A minimum of four different conformers of CSP 2 may be present due to the rotation of single bonds which are adjacent to the carbon–carbon double bond. In addition, the *trans*-CSP 1 could not form intramolecular hydrogen bonding, whereas the *cis*-CSP 2 could. The poorer performance of CSP 2 can be attributed to the conformational heterogeneity and competing intramolecular hydrogen bonding.
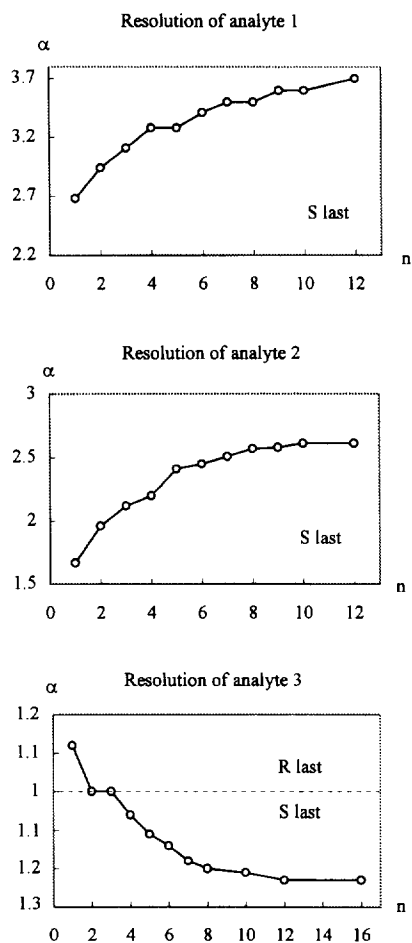
3.6. Resolution of homologous series of analytes on CSPs D and E

The information of the chiral recognition process could also be obtained by the investigation of the chiral resolution of homologous series on CSPs D and E which structures are similar to CSPs 1 and 2. The relationships of the separation factor and alkoxyl chain length are demonstrated in Table 3 and Fig. 8 and Fig. 9. The trends of enantioselectivities on CSP D (Fig. 8) were similar to those on CSP 1 (Fig. 5). An increase in enantioselectivity for the resolution of
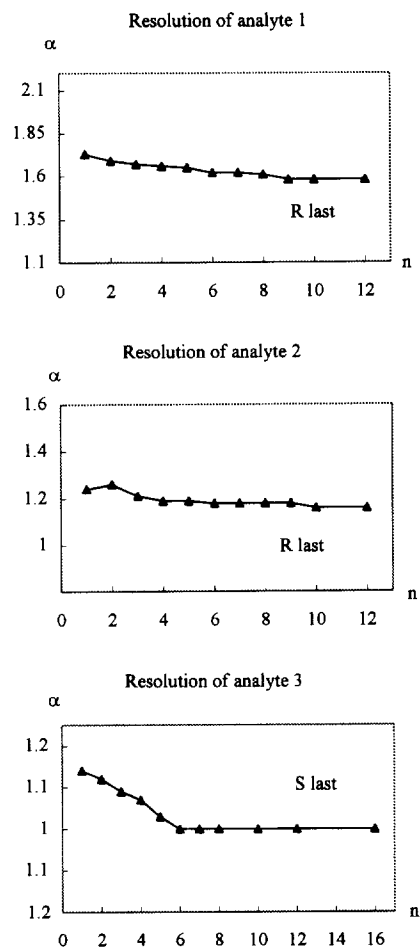

Fig. 8. Chiral resolution of homologous series on (S)-CSP D. Mobile phase: n-hexane–2-propanol (60:40) for the resolution of analytes 1 and 2, n-hexane–dichloromethane (50:50) for the resolution of analyte 3.

Fig. 9. Separation of enantiomeric homologues on (R)-CSP E. Mobile phase: n-hexane–2-propanol (80:20) for the resolution of analyte 1, n-hexane–2-propanol (70:30) for the resolution of analytes 2 and 3.

analytes 1 and 2 and a reversed of elution order for the resolution of analyte 3 were observed on CSP D. Thus, the chiral recognition models on CSP D for the resolution of analytes 1–3 were similar to models A and B. On the other hand, the trends of enantioselectivities on CSP E (Fig. 9) were similar to those on CSP 2 (Fig. 6). A decrease in enantioselectivity was observed for the resolution of analytes 1–3 on CSP E. The detailed chiral recognition process for the resolution of amino acids on the *ortho*-CSP E and the corresponding *para*-CSP will be discussed in the forthcoming paper [19]. However, the chromatographic results on CSP D and E for the resolution of homologous analytes 1–3 support the proposed chiral recognition models in this study.

4. Conclusions

The *trans*-CSP showed better chiral recognition ability than the *cis*-CSP for the resolution of the most enantiomers chosen in this study. The relationship between the separation factor and the alkoxyl chain length of amino acid alkyl ester depended upon the components of the mobile phase. Using *n*-hexane–dichloromethane as a mobile phase, a higher magnitude of difference between the *trans*-CSP and the *cis*-CSP in enantioselectivity was observed for the resolution of the three homologous series derived from amino acids. According to separation factors, the *trans*-CSP also showed better enantioselectivity for the resolution of homologues. This study clearly indicates that the chiral resolution is influenced by the geometry of the double bond in a CSP.

Acknowledgments

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