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Enantioseparation of amino acids and amino alcohols on chiral stationary phases derived from α-amino acid- and pyrrolidinyl-disubstituted cyanuric chloride

Ching-Erh Lin*, Chen-Hsing Lin, Fang-Kuo Li

Department of Chemistry, National Taiwan University, Roosevelt Road Section 4, Taipei 10764, Taiwan

Abstract

Three silica-based chiral stationary phases (CSPs) derived from L-α-amino acid- and pyrrolidinyl-disubstituted cyanuric chloride were prepared. The α-amino acids norvaline, valine, and phenylalanine were selected as the chiral moiety of CSPs. These CSPs provide effective recognition and separation of enantiomers of both methyl esters of N-(3,5-dinitrobenzoyl) amino acids, except that of proline, and N-(3,5-dinitrobenzoyl) amino alcohols by high-performance liquid chromatography. Amino acids, except phenylglycine, were generally separated more effectively than amino alcohols. Separation factors obtained for norephedrine on these CSPs, except CSP-2, are the best among reported values. Although the enantioselectivity depends mainly on three preferential interactions described previously which include a π–π interaction and two hydrogen-bonding interactions, steric interaction between substituents attached to chiral centers of chiral selectands and chiral selectors plays a significant role in the mechanism of chiral recognition. The phenyl ring in the phenylalanyl moiety of CSP-3 neither plays an electronic role in chiral recognition nor makes a significant contribution to chiral recognition. Comparison of chromatographic results shows that a CSP of a covalent type was more effective than the corresponding ionic type.

1. Introduction

Enantiomers of racemic mixtures can be efficiently and effectively separated on chemically bonded chiral stationary phases (CSPs) in a high-performance liquid chromatograph [1–3]. CSPs of various types have been designed for, and find application in, chiral separation. Among them, a Pirkle-type CSP based on the concept of three-point interaction is attracting great attention.

The mechanism by which enantiomers separate in the presence of a CSP is believed to be the formation of transient diastereomeric complexes between the chiral selectand and the chiral selector. The extent of enantioseparation may reflect differences in the energy of formation between the two transient diastereomeric complexes. The larger the energy difference, the better the enantioseparation.

Understanding the mechanism of chiral recognition is important for two reasons. First, it will afford insight into the factors governing the selection of an effective CSP to improve the resolution of a given analyte. Second, it will be a valuable aid in the design of a CSP with enhanced enantioselectivity. A large number of experimental [4–13] and/or computational [14–23] methods are being implemented, therefore, with the aim of eliciting wide-ranging information about the origins of chiral recognition.

* Corresponding author.

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For good enantioseparation to be achieved, the enantiomers to be separated must be structurally complementary to the chiral selector. There should be at least three sites of interaction between a chiral selectand and a chiral selector. This means that appropriate derivatives of analytes are typically necessary and may become a crucial factor for effective enantioseparation. As the *s*-triazine ring acts not only as a linking unit, introducing other functions in the chiral selector, but also as a π-basic unit capable of participating in $\pi-\pi$ interaction with an analyte carrying a π-acidic unit [24], the dinitrobenzoyl (DNB) group [25–32] generally serves as the π-acidic unit in chiral analytes.

We have previously described the preparation and HPLC performance of a silica-based chiral stationary phase derived from cyanuric chloride with an L-alanyl and a pyrrolidinyl substituent [24]. This CSP showed excellent enantioselectivity towards racemates of amino acids, with the exception of proline. Though the $\pi-\pi$ interaction and the two hydrogen bonds between chiral analytes and the CSP are considered to be the three major interactions between the chiral selector and chiral selectands, it is possible that the hydrogen bond between a secondary amino group of the chiral analytes and a carbonyl group of the chiral stationary phase is not essential for chiral discrimination. Rather, steric interaction between the substituent attached to the chiral center of the chiral selectands and the methyl group linked to the chiral center of the CSP is expected to play a significant role in chiral discrimination.

Here we report the preparation and chromatographic results of enantioseparation of amino acids and amino alcohols on three silica-based CSPs derived from L-α-amino acid- (L-norvaline-, L-valine-, or L-phenylalanine-) and pyrrolidinyl-disubstituted *s*-triazine derivatives, with the CSP reported previously [24]. We compare our chromatographic results with those, reported previously, obtained on the corresponding ionic-type CSPs [33]. These results clarify certain aspects, thus improving the model of chiral recognition.

2. Experimental

2.1. Chemicals and reagents

Pyrrolidine, cyanuric chloride, N,N′-dicyclohexylcarbodiimide and 3,5-dinitrobenzoyl chloride were purchased from Merck (Germany). The silica gel used was Nucleosil (pore size 10 nm; particle size 10 μm; surface area 350 m^2/g; supplied by Macherey-Nagel, Germany). 3-Aminopropyltriethoxysilane (APS) and N-methylmorpholine (Janssen, Belgium), N-hydroxysuccinimide (Aldrich, USA), amino acids (Sigma, USA), reagents for the chiral stationary phase and derivative of chiral analytes (from various suppliers) were used without further purification. 2-Propanol and hexane (Mallinckrodt, USA) were of LC grade. Water was purified with an ion exchanger and a Milli-Q water purification system (Millipore, USA).

2.2. Preparation of chiral stationary phases

2,4-Dichloro-6-pyrrolidinyl-s-triazine

The preparation of 2,4-dichloro-6-pyrrolidinyl-*s*-triazine has been described previously [24].

N-(2-Chloro-6-pyrrolidinyl-s-triazinyl)-L-amino acids

The L-amino acids norvaline, valine, and phenylalanine were used to prepare N-(2-chloro-6-pyrrolidinyl-*s*-triazinyl)-L-amino acids. The procedures to prepare N-(2-chloro-6-pyrrolidinyl-*s*-triazinyl)-L-amino acids were the same as that used for N-(2-chloro-6-pyrrolidinyl-*s*-triazinyl)-L-alanine described previously [24], except that L-alanine was replaced by the corresponding L-amino acid.

Silane-modified silica gels

Their preparation has been described previously [34]. The silane used is 3-aminopropylsilane.

Chemically bonded chiral stationary phase

After N-hydroxylsuccinimide (5 mmol) had been added to a solution of N-(2-chloro-6-

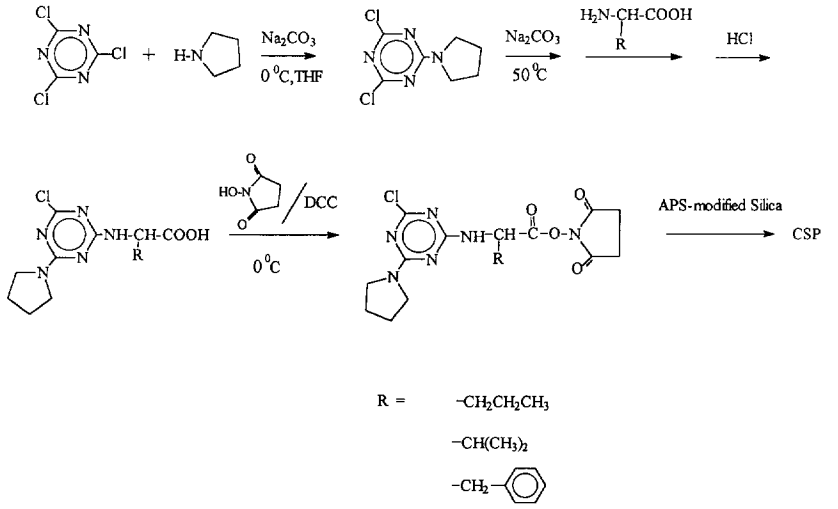


Fig. 1. Reaction schemes for the preparation of chiral stationary phases.

pyrrolidinyl-*s*-triazinyl)-L-amino acid (5 mmol) dissolved in tetrahydrofuran (THF, 100 ml) in an ice bath at 0°C, N,N′-dicyclohexylcarbodiimide (DCC, 5 mmol) was added slowly, with agitation. Reaction proceeded at 0°C for 1 h, then at room temperature for a further 24 h. The reaction product (in THF solution) was obtained by filtering off unwanted dicyclohexylurea. Then APS-modified silica gel (3 g) and N-methylmorpholine (1 ml) were added to this THF solution. The reaction proceeded at 0°C for 1 h, then at room temperature for a further 48 h with agitation. The product was collected by filtration, washed thoroughly with THF, methanol, water, and methanol successively, then dried over P_2O_5 at reduced pressure. Fig. 1 presents reaction schemes for the preparation of these chiral stationary phases. Fig. 2 depicts the structures of the CSPs prepared.

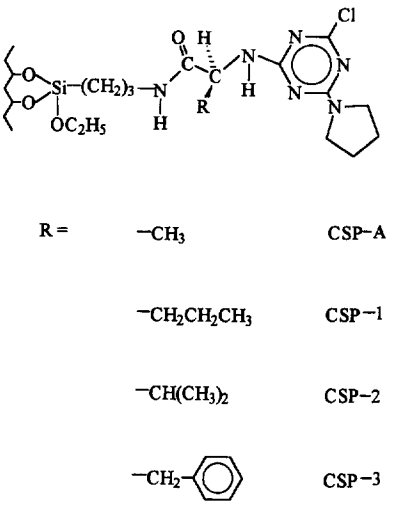


Fig. 2. Structures of chiral stationary phases prepared.

2.3. Apparatus and chromatography

The chromatographic system and column packing were as described previously [33]. Mixtures of 2-propanol and hexane were used as the mobile phase and were filtered through a membrane filter (0.45 μm) and degassed under ultrasonic vibration. The flow-rate was 1.0 ml/min; the detector was operated at 254 nm. Elemental analyses of the chiral stationary phase and its corresponding silane-modified silica gel were performed with an elemental analyzer (Perkin-Elmer Model 240C).

Table 1
Characteristics of 3-aminopropylsilica and the CSPs

Sample	Elemental analysis (N%)	Loading capacity (mmol/g)
3-Aminopropylsilica	1.29	0.92
CSP-A	3.31	0.33
CSP-1	3.24	0.32
CSP-2	2.91	0.26
CSP-3	2.81	0.25

3. Results and discussion

3.1. Characterization of chiral stationary phases

Table 1 presents the nitrogen content of chiral stationary phases obtained from elemental analyses and the loading capacities of chiral stationary phases determined from the nitrogen content. According to the equation described previously [24], the loading capacities of CSP-1–CSP-3 are in the range 0.25–0.33 mmol/g. Thus only 27–36% of the amino groups on the APS-modified silica surface were converted into the chiral moiety.

3.2. Enantioseparation of amino acids

Table 2 presents chromatographic results of enantiomeric separation of methyl esters of N-(3,5-dinitrobenzoyl) amino acids on CSP-1–CSP-3, with results on CSP-A [24] for comparison. These phases provide excellent recognition and separation of enantiomers of methyl esters of N-(3,5-dinitrobenzoyl) amino acids. In most instances, enantiomers of amino acids were completely separated with 2-propanol–hexane (20:80 or 15:85, v/v) as eluent. Fig. 3 shows typical chromatograms of the enantiomeric separation of the methyl ester of N-(3,5-dinitrobenzoyl)valine on CSP-1–CSP-3.

The trends in the variation of capacity factors and enantioselectivities of enantiomers of amino acids on CSP-1 and CSP-2 were similar to those observed for CSP-A (Table 2). The enantioselectivities of amino acids on CSP-2 were lower than those on CSP-1 and CSP-A. The presence of a bulkier valyl group in CSP-2 is apparently unfavorable for chiral recognition. This result may also imply that a steric effect on chiral separation is significant.

Table 2
Chromatographic results of the enantiomeric separation of N-(3,5-dinitrobenzoyl) amino acids on CSPs

Solute	CSP-A			CSP-1			CSP-2			CSP-3		
	k_1'	α	MP	k_1'	α	MP	k_1'	α	MP	k_1'	α	MP
Alanine	3.65	1.36	a	3.50	1.36	a	3.70	1.20	a	5.14	1.33	b
Aminobutyric acid	2.78	1.45	a	4.18	1.45	b	2.81	1.25	a	3.95	1.44	b
Norvaline	2.31	1.39	a	3.19	1.40	b	2.31	1.24	a	2.99	1.42	b
Norleucine	2.09	1.35	a	2.90	1.37	b	2.09	1.22	a	1.94	1.39	a
Valine	2.38	1.54	a	2.12	1.54	a	3.05	1.30	b	2.79	1.57	b
Leucine	2.01	1.44	a	1.82	1.46	a	2.48	1.29	b	1.78	1.46	a
Isoleucine	2.03	1.49	a	1.97	1.51	a	2.52	1.30	b	1.87	1.51	a
Phenylglycine	4.46	1.20	a	5.78	1.19	b	4.80	1.13	b	5.49	1.22	b
Phenylalanine	3.95	1.53	a	3.49	1.52	a	4.60	1.35	b	3.60	1.43	a
Tryptophan	11.89	1.55	a	11.05	1.63	a	10.20	1.43	a	10.96	1.51	a
Aspartic acid	6.43	1.28	a	8.79	1.31	b	9.51	1.16	b	6.49	1.33	a
Glutamic acid	5.85	1.40	a	7.95	1.38	b	9.04	1.25	b	5.92	1.42	a
Methionine	5.02	1.46	a	4.69	1.49	a	6.26	1.30	b	4.59	1.44	a
Threonine	6.53	1.17	a	9.08	1.21	b	6.08	1.14	a	9.89	1.21	b
Proline	3.45	1.00	a	3.90	1.00	b	3.04	1.00	a	3.94	1.00	b

The absolute configuration of the first-eluted enantiomer is the R-form; k_1' is the capacity factor of the first-eluted enantiomer; flow-rate 1 ml/min; MP denotes the composition of the mobile phase (2-propanol–*n*-hexane, v/v): a = 20:80; b = 15:85.

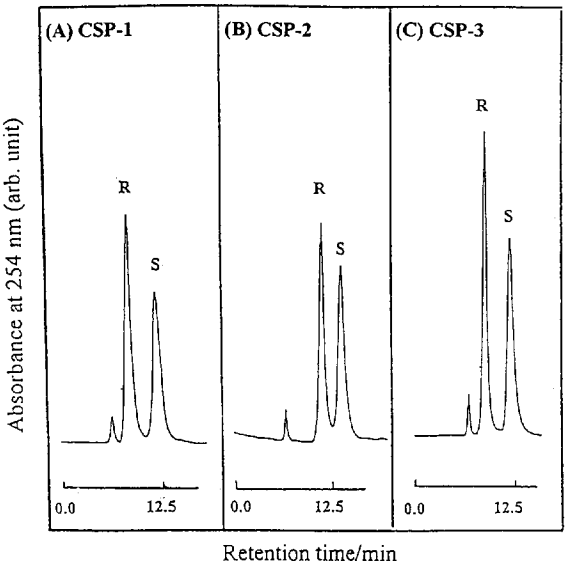


Fig. 3. Chromatograms of enantioseparation of the methyl ester of N-(3,5-dinitrobenzoyl)valine on CSPs. (A) CSP-1, (B) CSP-2, and (C) CSP-3. (A) Eluent: 2-propanol–*n*-hexane (20:80, v/v); flow-rate 1 ml/min; (B) and (C) eluent: 2-propanol–*n*-hexane (15:85, v/v), flow-rate 1 ml/min.

As the α-values of amino acids on CSP-3 are generally similar to those of amino acids on CSP-1 and CSP-A, the role played by the phenylalanyl moiety of CSP-3 in chiral recognition is evidently similar to that played by the norvalyl moiety of CSP-1 and by the alanyl moiety of CSP-A. In particular, as chromatographic results obtained for phenylglycine and phenylalanine on CSP-1 resemble those on CSP-3, which has a benzyl group on the chiral selector, the possibility of an additional $\pi-\pi$ interaction between the benzyl group of the chiral selectand and that of the chiral selector is excluded. Hence the phenyl ring in the phenylalanyl moiety of CSP-3 has no electronic role in the recognition of racemates of amino acids by the chiral selector on CSP-3, which is consistent with the findings reported previously [35].

The α-values for phenylglycine, phenylalanine, and tryptophan are 1.19, 1.52, and 1.63, respectively, on CSP-1, but 1.22, 1.43, and 1.51, respectively, on CSP-3. The remarkably varied enantioselectivity between phenylglycine and phenylalanine or tryptophan on both CSP-1 and CSP-3 may be related to differences in the rigidity of the conformation of chiral analytes depending on the aromatic substituent(s) attached to the chiral center.

Features were observed for amino acids with a carboxyl group, such as aspartic and glutamic acids, on CSP-1 and CSP-3 similar to those found previously on CSP-A. Comparison of chromatographic results for threonine with those for valine on these CSPs reveals that the formation of a hydrogen bond between the hydroxyl group of threonine and the CSP produces more non-enantioselective interactions. This hydrogen bond may compete or interfere with the other hydrogen bond, the formation of which, between the secondary amino group in chiral selectands and the carbonyl group in the chiral selector, is considered to be a major preferential interaction responsible for chiral recognition.

It may be assumed that the hydroxyl group of threonine forms a hydrogen bond with the unreacted amino group of the APS-modified silica. This hydrogen bond is non-enantioselective and simply increases the retention of both enantiomers of threonine. In consequence, the separation factor of threonine might be smaller than that of valine, whereas the capacity factor is larger than that of valine. As the α-value of threonine is smaller than that of valine, the presence of the hydroxyl group in threonine is unfavorable for chiral discrimination.

The capacity factors and enantioselectivities obtained for amino acids with a bifunctional aromatic substituent, such as tryptophan, are considerably enhanced. Hence, an additional interaction between chiral selectand and chiral selector is involved.

The enantiomers of N-(3,5-dinitrobenzoyl)-derivatized proline were not resolved on all CSPs tested; the reason is most likely the absence of an acidic NH in the proline derivative. This would mean that the lack of a binding site to form a hydrogen bond prevents enantioseparation from taking place.

Ionically bonded CSPs derived from L-amino acid- and pyrrolidinyl-disubstituted cyanuric chloride also provide the ability to recognize and

separate enantiomers of amino acids and amino alcohols [33]. However, the α-values obtained on these ionic-type CSPs were less than 1.22. From this comparison we conclude that CSPs of covalent types are much more effective than their ionic counterparts.

3.3. Enantioseparation of amino alcohols

Table 3 presents results of enantiomeric separation of N-(3,5-dinitrobenzoyl) amino alcohols on CSP-A and CSP-1–CSP-3. Fig. 4 shows typical chromatograms of the enantiomeric separation of N-(3,5-dinitro-benzoyl)valinol on CSP-1–CSP-3. In general, chiral analytes were retained longer than their amino acid counterparts on all CSPs tested. These amino alcohols, with the exception of phenylglycinol, for which the α-value is substantially larger than that for phenylglycine, exhibited larger capacity factors owing to greater non-enantioselective interaction, but smaller α-values than those of amino acids on the same column. For instance, the α-values of alaninol and alanine were 1.33 and 1.36 on CSP-A, but 1.27 and 1.36 on CSP-1, 1.10 and 1.20 on CSP-2, and 1.30 and 1.33 on CSP-3, respectively. As $\pi-\pi$ interaction involving the 3,5-dinitrobenzoyl group of the chiral selectand and the pyrrolidinyl-substituted *s*-triazine ring of the CSP, and hydrogen bonding involving the secondary amino group in the amide linkage of the chiral selectand and the carbonyl group in the amide linkage of the chiral selector, are essentially the same in both amino acids and amino alcohols, these results indicate that the hydrogen-bonding interaction between the CSP and the

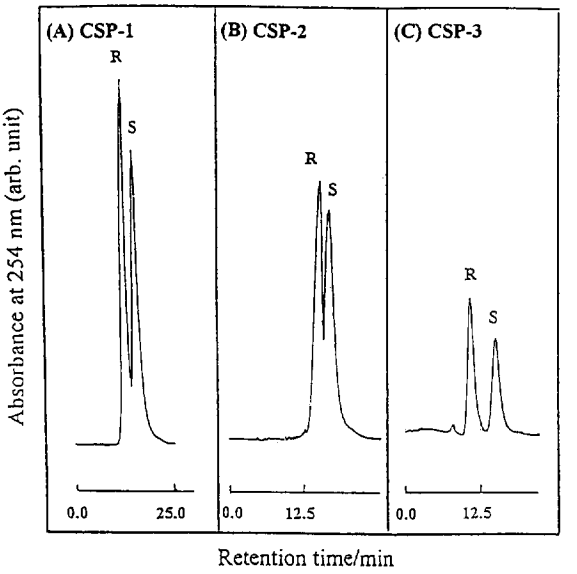


Fig. 4. Chromatograms of enantioseparation of N-(3,5-dinitrobenzoyl)valinol on CSPs. (A) CSP-1, (B) CSP-2, and (C) CSP-3. (A) Eluent: 2-propanol–*n*-hexane (15:85, v/v); flow-rate 1 ml/min; (B) and (C) eluent: 2-propanol–*n*-hexane (20:80, v/v); flow-rate 1 ml/min.

Table 3
Chromatographic results of the enantiomeric separation of N-(3,5-dinitrobenzoyl) amino alcohols on CSP-A, CSP-1, CSP-2, and CSP-3

Solute	CSP-A			CSP-1			CSP-2			CSP-3		
	k_1'	α	MP	k_1'	α	MP	k_1'	α	MP	k_1'	α	MP
Alaninol	5.25	1.33	a	6.84	1.27	b	5.25	1.10	a	5.41	1.30	a
2-Amino-1-butanol	3.55	1.36	a	4.56	1.30	b	3.92	1.12	a	3.53	1.34	a
Valinol	2.03	1.52	c	3.91	1.35	b	3.04	1.15	a	2.94	1.49	a
Phenylglycinol	7.05	1.42	a	6.87	1.34	a	7.01	1.16	a	7.17	1.50	a
Phenylalaninol	5.14	1.41	a	5.09	1.30	a	5.15	1.14	a	5.31	1.39	a
Norephedrine	3.12	1.68	c	5.11	1.69	b	5.53	1.37	b	3.59	1.76	a

The absolute configuration of the first-eluted enantiomer is the R-form; k_1' is the capacity factor of the first-eluted enantiomer; flow-rate 1 ml/min; MP denotes the composition of the mobile phase (2-propanol–*n*-hexane, v/v): a = 20:80; b = 15:85; c = 30:70.

ester group of chiral selectands in amino acids is greater than that between the CSP and the hydroxyl group of chiral selectands in amino alcohols.

Trends in the variation of capacity factors of enantiomers of amino alcohols were similar to those for amino acids on the same CSP. The smaller capacity factors and the larger α-values of valinol on CSP-A and CSP-1–CSP-3 compared with those of alaninol and 2-amino-1-butanol indicate that the greater the steric interaction due to the presence of a bulkier group in the chiral selectand (or to the structural rigidity of the conformation), the better the enantioseparation.

Chiral recognition was excellent for phenylglycinol and phenylalaninol on CSP-A, CSP-1, and CSP-3, but only good on CSP-2. As the chromatographic results for these two chiral analytes on CSP-3 are similar to those on CSP-A and CSP-1, interactions between the chiral selector in CSP-3 and the chiral selectands of these two analytes are correspondingly similar to those between the chiral selectors in both CSP-A and CSP-1 and the two chiral analytes. As for phenylglycine and phenylalanine mentioned earlier, the results indicate that the phenyl ring in the phenylalanyl moiety of the CSP makes no significant contribution to chiral recognition and does not play an electronic role in the chiral recognition.

No enantioseparation of these two analytes was achieved on CSPs of the corresponding ionic type [33], despite the three preferential interactions responsible for chiral recognition still existing.

CSP-1–CSP-3 and CSP-A are excellent at recognizing and separating enantiomers of norephedrine, which is an amino alcohol with two adjacent chiral centers. The α-values obtained for norephedrine on CSP-1–CSP-3 and CSP-A are 1.69, 1.37, 1.76, and 1.68, respectively. The α-values obtained for norephedrine on these CSPs, with the exception of CSP-2, are much larger than reported values [36–39]. Fig. 5 shows enantiomeric separation chromatograms for 3,5-dinitrobenzoyl-derivatized norephedrine on CSP-1–CSP-3.

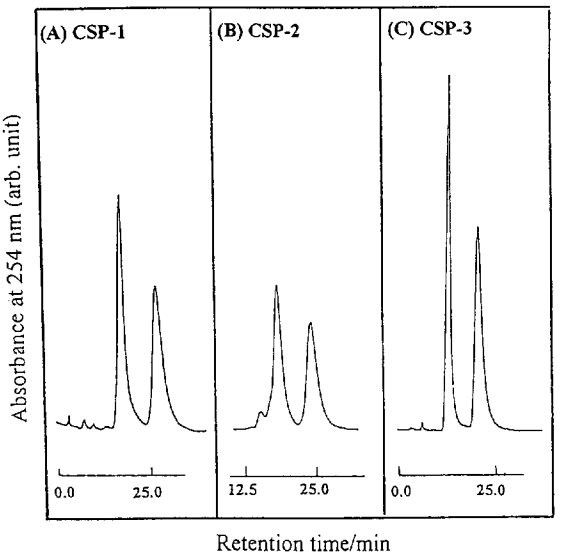


Fig. 5. Chromatograms of the enantioseparation of N-(3,5-dinitrobenzoyl)norephedrine on CSPs. (A) CSP-1, (B) CSP-2, and (C) CSP-3. (A) and (B) Eluent: 2-propanol–*n*-hexane (15:85, v/v); flow-rate 1 ml/min; (C) eluent: 2-propanol–*n*-hexane (20:80, v/v); flow-rate 1 ml/min.

3.4. Chiral recognition mechanism

As described previously [24], the mechanism of chiral recognition of the CSPs tested is based mainly on three preferential interactions, including a $\pi-\pi$ interaction and two hydrogen-bonding interactions between chiral selectands and chiral selectors. However, these preferential interactions may not be the only factors determining chiral recognition. Additional interactions, such as steric interaction between the substituents attached to the chiral center of the chiral selectand and the chiral center of the chiral selector, may play a significant role in chiral discrimination. For instance, under conditions whereby hydrogen bond formation, considered a preferential interaction, was eliminated, enantiomers of N-methylalanine were barely resolved on an ionic-type CSP containing an alanyl chiral moiety [33].

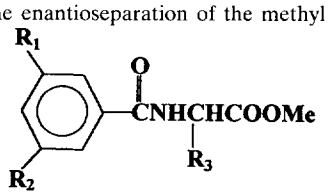
The fact that a $\pi-\pi$ interaction involving the 3,5-dinitrobenzoyl group in a chiral selectand and the pyrrolidinyl-substituted *s*-triazine ring in a

chiral selector is essential for chiral recognition has been concluded from a previous investigation [24]. To confirm the role of an interaction of this sort, further chromatographic results relating to the enantioseparation of methyl esters of benzoyl-derivatized amino acids with various acceptor substitutents on CSP-1 and CSP-3 are provided. Table 4 presents such results for the enantioseparation of methyl esters of various methionine derivatives and of methyl esters of valine derivatives on CSP-1 and CSP-3. Replacement of a 3,5-dinitrobenzoyl group by a hydrogen or chloride atom evidently decreases the enantioselectivity. This effect is simply due to the decreased $\pi-\pi$ interaction between the *s*-triazine ring of the chiral selector and the benzoyl group of the chiral selectand. Our chromatographic data thus confirm the necessity of the presence of a pyrrolidinyl-*s*-triazine ring in the chiral selector.

The important role played by the ester group of chiral analytes in chiral discrimination has been demonstrated previously [40,41]. By comparing chromatographic results for 3,5-dinitrobenzyol derivatives of amino acids with those for the corresponding 3,5-dinitrobenzoyl amine [24], additional evidence to support the important role of this carboxyl group was obtained. The absence of the carbonyl group in 3,5-dinitrobenzoylamine would eliminate a site for hydrogen-bond formation, considered to be a preferential interaction. Hence, chiral discrimination was not achieved for 3,5-dinitrobenzoyl amine. Smaller α-values were generally observed for 3,5-dinitrobenzoyl derivatives of amino alcohols compared with the α-values of the corresponding amino acids. Thus, the replacement of the carboxyl group of amino acids by the hydroxyl group of amino alcohols decreases the ability to form a hydrogen bond.

The enantioselectivity of amino acids is greatly affected by the bulkiness, or chain length, of an alkyl ester group of a chiral selectand. Table 5 presents chromatographic results for enantiomers of various alkyl esters of 3,5-dinitrobenzoyl derivatives of methionine and valine on CSP-1 and CSP-3. In all instances, when the ester group of amino acids is changed from methyl ester to isopropyl ester or pentyl ester, the capacity factors of the amino acid esters decrease substantially, whereas the enantioselectivity increases considerably. For example, on CSP-1, the α-values are 1.54, 1.72, 1.75, and 1.77 for the methyl ester, the butyl ester, the pentyl ester, and the isopropyl ester of N-(3,5-dinitroben-

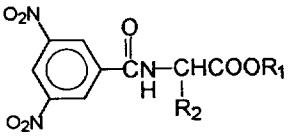
Table 4
Effect of various π-acceptor substituents on the enantioseparation of the methyl esters of benzoyl-derivatized amino acids



R_1	R_2	CSP-1				CSP-3			
		$R_3 = CH_2CH_2SCH_3$		$R_3 = CH(CH_3)_2$		$R_3 = CH_2CH_2SCH_3$		$R_3 = CH(CH_3)_2$	
		k_1'	α	k_1'	α	k_1'	α	k_1'	α
NO_2	NO_2	6.92	1.47	2.99	1.54	6.75	1.43	2.79	1.57
H	NO_2	4.98	1.09	2.14	1.11	5.23	1.12	2.21	1.13
H	Cl	2.01	1.00	0.84	1.00	1.93	1.06	0.80	1.00
H	H	2.13	1.00	0.86	1.00	2.33	1.00	0.82	1.00

The absolute configuration of the first-eluted enantiomer is the R-form; k_1' is the capacity factor of the first-eluted enantiomer; flow-rate 1 ml/min; eluent: 2-propanol–*n*-hexane (20:80, v/v).

Table 5
Chromatographic results for the enantiomers of various alkyl esters of 3,5-dinitrobenzoyl-derivatized amino acids on CSP-1 and CSP-3



R_1	CSP-1				CSP-3			
	$R_2 = CH_2CH_2SCH_3$		$R_2 = CH(CH_3)_2$		$R_2 = CH_2CH_2SCH_3$		$R_2 = CH(CH_3)_2$	
	k_1'	α	k_1'	α	k_1'	α	k_1'	α
CH_3-	6.92	1.47	2.99	1.54	4.59	1.44	2.11	1.54
$CH_3(CH_2)_2-$	3.75	1.66	1.87	1.72	2.66	1.56	1.35	1.64
$CH_3(CH_2)_4-$	2.91	1.70	1.55	1.75	2.15	1.60	1.11	1.70
$(CH_3)_2CH-$	3.31	1.74	1.68	1.77	2.31	1.58	1.20	1.72

The absolute configuration of the first-eluted enantiomer is the R-form; k_1' is the capacity factor of the first-eluted enantiomer; flow-rate 1 ml/min; eluent: 2-propanol–*n*-hexane (20:80, v/v).

zoyl)valine, respectively. The enhanced enantioselectivity of the isopropyl ester of N-(3,5-dinitrobenzoyl)valine resulting from the steric effect is demonstrated clearly.

The inability to resolve enantiomers of proline on the CSPs tested, and to resolve enantiomers of N-methylalanine on their ionic counterparts [33], appears to be due to the absence of an acidic NH group in these analytes. Under these conditions, no hydrogen bond was formed between the chiral analyte and the chiral selector; thus no enantioseparation was achieved. The implication is that the secondary amino group in chiral analytes also plays an important role in chiral discrimination.

As similar trends in the variation of capacity factors and selectivity factors were observed for N-(3,5-dinitrobenzoyl) amino acids and N-(3,5-dinitrobenzoyl) amino alcohols, the mechanism of chiral recognition is expected to be similar for amino acids and amino alcohols.

4. Conclusions

Chiral stationary phases derived from L-amino acid- and pyrrolidinyl-disubstituted cyanuric chloride provide effective enantioselectivities for most tested racemates of amino acids, with the exception of proline, and amino alcohols.

The α-values for norephedrine on these CSPs, except CSP-2, are much larger than reported values.

Although the recognition ability is mainly based on three preferential interactions, a $\pi-\pi$ interaction and the formation of two hydrogen bonds, steric interaction between substituents attached to chiral centers of the chiral selector and chiral selectands also plays a significant role in chiral discrimination.

The phenyl ring in the phenylalanyl moiety of CSP-3 neither plays an electronic role in chiral recognition nor makes a significant contribution to chiral recognition.

Chiral analytes are separated more effectively on a CSP of a covalent type than on the corresponding ionic type.

The results of this work enhance insight into chiral recognition, thereby improving the chiral recognition model.

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