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Enantiomer separation of amino acids on a chiral stationary phase derived from L-alanyl- and pyrrolidinyl-disubstituted cyanuric chloride

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Abstract

A silica-based chiral stationary phase derived from L-alanyl- and pyrrolidinyl-disubstituted cyanuric chloride was prepared for the enantioseparation of methyl esters of N-(3,5-dinitrobenzoyl)amino acids. The chromatographic results show that effective and efficient enantioseparation was achieved on this chiral stationary phase. Rationales purporting to account for liquid chromatographic observations of chiral recognition are examined.

1. Introduction

Owing to the increasing awareness of the importance of separating enantiomers, interest in the separation of enantiomers by high-performance liquid chromatography has grown dramatically during the past decade [1–5]. The efficient separation and resolution of enantiomers from racemic mixtures can be achieved by utilizing chemically bonded chiral stationary phases (CSPs) [6–9]. For the successful resolution of enantioseparation on CSPs, the molecular structures of the chiral moieties of the CSP and the enantiomeric analytes must be complementary in some fashion so that at least three simultaneous, preferential and distinct interactions prevail between them [10]. A CSP capable of preferentially retaining one of a pair of enantiomers should exhibit at least one preferential interaction that is stereochemically dependent. The preferential interactions important for enantioselectivity cause a differential retention of the enantiomeric analytes. As the extent of these interactions depends on the molecular structures of enantiomeric analytes, an appropriate derivatization of chiral molecules may become a determining factor for the effective and selective resolution of enantiomeric analytes.

The design of effective and selective CSPs is often aided by understanding the mechanism of chiral recognition. Recently, the origin of chiral recognition has been investigated by many researchers using empirical [11–20] and/or computational [21–29] methods. In this way, chiral recognition models pertaining to the mode of operation of those CSPs become better understood.

As the high-performance liquid chromatographic separation of enantiomers on some *s*-triazine derivatives of amino acid dipeptide es-

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ters, tripeptide esters and (*S*)-1-(α-naphthyl)-ethylamine chiral stationary phases [30–32] demonstrated the practicability of using the *s*-triazine ring as a linking unit, we considered that in the design of new chiral stationary phases the chiral selector can be composed of an amino acid and an *s*-triazine derivative possessing strong π-basic character for the chiral separation of amino acids. In this work, a CSP composed of an L-alanyl- and pyrrolidinyl-disubstituted *s*-triazine derivative was prepared and the enantioselectivities of some amino acids on this CSP column were examined. Rationales purporting to account for liquid chromatographic observations of chiral recognition were considered.

2. Experimental

2.1. Chemicals and reagents

Pyrrolidine, cyanuric chloride, N,N-dicyclohexylcarbodiimide and 3,5-dinitrobenzoyl chloride were purchased from Merck. The silica gel used was Nucleosil (pore size 10 nm; particle size 10 mm; surface area 350 m^2/g) obtained from Macherey–Nagel. 3-Aminopropyltriethoxysilane (APS) and N-methylmorpholine were obtained from Janssen. N-Hydroxysuccinimide was purchased from Aldrich and amino acids from Sigma. Synthesis reagents for preparing the chiral stationary phase and the derivatization of chiral analytes were purchased from various suppliers and were used as received. 2-Propanol and *n*-hexane of LC grade were purchased from Mallinckrodt. Water was purified by ion exchange followed by treatment in a Milli-Q water purification system (Millipore).

2.2. Preparation of chiral stationary phase

Pyrrolidinyl-substituted cyanuric chloride

A solution of sodium carbonate (0.021 mol) and pyrrolidine (0.02 mol) in water (70 ml) kept in an ice-bath near 0°C was added with agitation to a solution of cyanuric chloride (0.02 mol) in acetone (30 ml). After the mixed solution had reacted at 0°C for 1 h, the product was filtered, washed well with cold water several times and then dried over P_4O_{10} at reduced pressure. The product yield is about 85%.

L-Alanyl- and pyrrolidinyl-disubstituted cyanuric chloride

A solution of sodium carbonate (0.021 mol) and L-alanine (0.01 mol) dissolved in water (100 ml) was added with agitation to a solution of pyrrolidinyl-substituted cyanuric chloride (0.01 mol) dissolved in acetone (30 ml). The mixed solution was reacted near 50°C for 2 h. The solution was filtered to remove the precipitate, then the clear filtrate was neutralized with dilute HCl solution until substantial precipitation occurred, with the filtrate in the ice-bath. The precipitate was collected by filtration, washed several times with water and then dried over P_4O_{10} at reduced pressure. The yield of the product was about 55%.

Silane-modified silica gels

The preparation of silane-modified silica gels was described previously [33]. The silane used was 3-aminopropylsilane.

Chemically bonded chiral stationary phase

After adding N-hydroxysuccinimide (0.005 mol) to a solution of L-alanyl- and pyrrolidinyl-disubstituted cyanuric chloride (0.005 mol) dissolved in tetrahydrofuran (THF) (100 ml) in an ice-bath at 0°C, N,N-dicyclohexylcarbodiimide (DCC) (0.005 mol) was slowly added with agitation. Reaction proceeded at 0°C for 1 h, then at room temperature for a further 24 h. The solution product was obtained by filtering off unwanted dicyclohexylurea, in which APS-modified silica gel (3 g) was suspended and to which N-methylmorpholine (1 ml) was added. 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_4O_{10} at reduced pressure. Fig. 1 displays the reaction scheme for the preparation of this chiral stationary phase.

2.3. Apparatus and chromatography

The chromatographic system and the column-packing apparatus were described previously [32]. Mixtures of 2-propanol and *n*-hexane (typi-

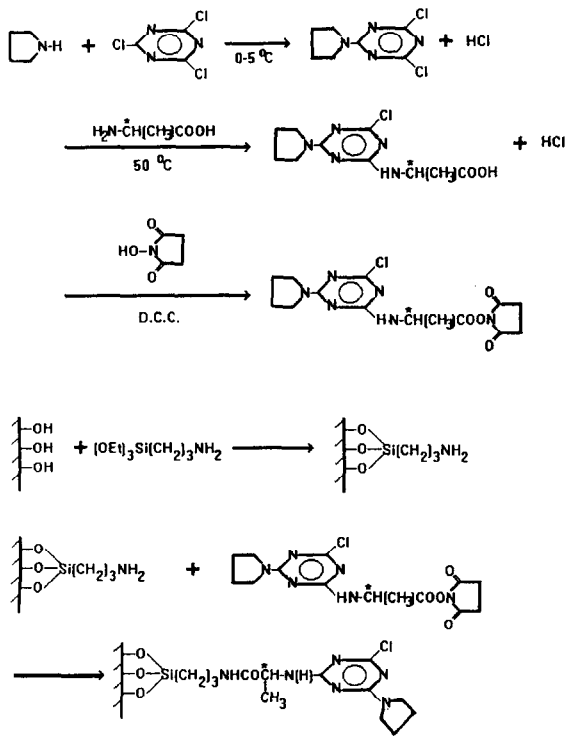


Fig. 1. Reaction scheme for the preparation of chiral stationary phase.

cally 20:80, v/v) were used as the mobile phase and were filtered through a 0.45-μm membrane filter and degassed by ultrasolic vibration. The flow-rate was 1.0 ml/min. The detector was operated at 254 nm. Elemental analyses of the chiral stationary phase and the corresponding silane-modified silica gel were performed with a Perkin-Elmer Model 240C elemental analyser.

3. Results and discussion

3.1. Characterization of the stationary phase

The nitrogen contents of APS-modified silica and chiral stationary phase obtained from elemental analyses were 1.29 and 3.31%, respectively. The loading capacities determined from the nitrogen contents for APS-modified silica and chiral stationary phase were 0.92 and 0.33 mmol/g, respectively. The loading capacity of APS-modified silica (X) was calculated with the equation

$$X\,(\text{mmol}) = \text{N}\,(\%) \times 1000/(14 \times 100)$$

and the loading capacity of the chiral stationary phase (Z, in mmol/g) was calculated with the equation

$$\text{N}\,(\%) = (14X + 5 \times 14Z) \times 10^{-3} \times 100/ [1 + (M) - 18)Z] \times 10^{-3}$$

where M is the molecular mass of pyrrolidinyl- and L-alanyl-disubstituted cyanuric chloride. By comparing the loading capacity of the APS-modified silica with that of the chiral entity, we found that only 36% of the amino groups on the APS-modified silica surface were converted into the chiral moiety.

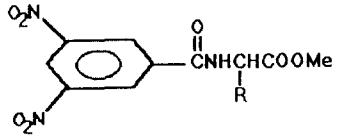
3.2. Enantioseparation of amino acids

Table 1 presents the results of the enantiomeric separation of methyl esters of 3,5-dinitrobenzoyl amino acids on this chiral stationary phase. Fig. 2 shows typical chromatograms of the enantiomeric separation of valine and leucine. Except for proline, which shows no chiral selectivity, most of the amino acids listed in Table 1 showed excellent enantioselectivity. In most instances, baseline separation of the enantiomers of amino acids was achieved using 2-propanol–*n*-hexane (20:80, v/v) as the eluent.

As shown in Table 1, the capacity factors of the enantiomers of amino acids having an alkyl substituent attached to the chiral carbon decrease with increasing chain length of the alkyl group, but the enantioselectivity indicated by the α values seems to be unaffected by variation of the chain length of the alkyl group. In contrast, the enantioselectivities of amino acids with a branched-chain alkyl group are larger than those of amino acids with a linear alkyl group. This may indicate that a small but stereochemically significant steric interaction exists between the branched-chain alkyl group of the chiral selectrand and the methyl group of the chiral selector.

Among the chiral analytes tested, the enantiomers of amino acids with substituents other than an alkyl group were retained longer in the

Table 1
Chromatographic separation of methyl esters of N-(3,5-dinitrobenzoyl)amino acids on L-alanyl- and pyrrolidinyl-disubstituted cyanuric chloride-derived CSP



Amino acid	R	k_1' [a]	α	Configuration[b]
Alanine	CH_3	3.65	1.36	*R*
Aminobutyric acid	CH_3CH_2	2.78	1.45	*R*
Norvaline	$CH_3CH_2CH_2$	2.31	1.39	*R*
Norleucine	$CH_3CH_2CH_2CH_2$	2.09	1.35	*R*
Valine	$(CH_3)_2CH$	2.38	1.54	*R*
Leucine	$CH_3CH(CH_3)CH_2$	2.01	1.44	*R*
Isoleucine	$CH_3CH_2CH(CH_3)$	2.03	1.49	*R*
Phenylglycine	C_6H_5	4.46	1.20	*R*
Phenylalanine	$C_6H_5CH_2$	3.95	1.53	*R*
Tyrosine	$HOC_6H_5CH_2$	10.78	1.62	*R*
Tryptophan	$C_8H_5(NH)CH_2$	11.89	1.55	*R*
Aspartic acid	CH_3OOCCH_2	6.63	1.28	*R*
Glutamic acid	$CH_3OOCCH_2CH_2$	5.85	1.40	*R*
Methionine	$CH_3SCH_2CH_2$	5.02	1.46	*R*
Threonine	$HOCH(CH_3)$	6.53	1.17	*R*
Proline	–	3.45	1.00	–

Eluent, 2-propanol–*n*-hexane (20:80, v/v); flow-rate, 1 ml/min.
[a] Capacity factor of the first-eluted enantiomer.
[b] Absolute configuration of the first-eluted enantiomer.

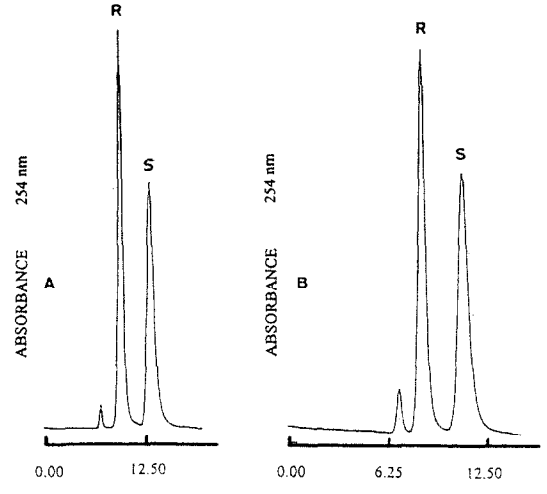


Fig. 2. Enantioseparation of methyl esters of N-(3,5-dinitrobenzoyl)amino acids: (A) valine; (B) leucine. Eluent, 2-propanol–*n*-hexane (20:80, v/v); flow-rate, 1 ml/min.

column than those of amino acids with alkyl substituents, particularly for amino acids with aromatic substituents such as tyrosine and tryptophan. Hence the involvement of the additional π–π interaction between amino acids with aromatic substituents and the chiral stationary phase is clear. As indicated in Table 1, the α values for phenylglycine, phenylalanine, tyrosine and tryptophan were 1.20, 1.53, 1.62 and 1.55, respectively. The remarkable difference in the enantioselectivity between phenylglycine and phenylalanine, tyrosine or tryptophan reveals that the relative orientation of the aromatic substituent and the structural compatibility between these chiral analytes and the chiral stationary phase plays a significant role in the enantioseparation.

The capacity factors of amino acids with a carboxyl group such as aspartic acid and glutamic acid esters were found to be twice as large as those of amino acids with an alkyl group. This

effect is probably due to hydrogen bonding between the carbonyl group of the chiral analytes and the secondary amino group of the chiral stationary phase. However, as reflected by the smaller α value of aspartic acid than that of amino acids with alkyl substituents, this interaction could not be considered as a preferential interaction contributing to the formation of diastereomeric complexes.

The structural difference between threonine and valine clearly indicates that the presence of a hydroxyl group in threonine causes an increased capacity factor, because of hydrogen bonding with the carbonyl group of the chiral stationary phase. However, as reflected by the smaller α value of threonine than that of valine, the existence of this interaction seems not to favour chiral recognition also.

The enantiomers of N-(3,5-dinitrobenzoyl)-derivatized proline molecule cannot be resolved on this chiral stationary phase. The reasons are unknown; perhaps the absence of an acidic NH group in the derivatized proline prevents hydrogen bonding.

3.3. Chiral recognition mechanism

In accordance with previous reports [11–13], the design of the present chiral stationary phase is based on the following three preferential interactions: (a) $\pi-\pi$ interaction involving the 3,5-dinitrobenzoyl group of the chiral selectrand and the pyrrolidinyl-substituted *s*-triazine ring of the CSP, (b) hydrogen bonding involving the carbonyl group in the carboxyl ester group of the chiral selectrand and the secondary amino group belonging to the amino acid of the chiral selector and (c) hydrogen bonding involving the secondary amino group in the amide linkage of the chiral selectrand and the carbonyl group in the amide linkage of the chiral selector. Fig. 3 shows the preferential interactions existing between the chiral selectrand and the chiral selector. However, as mentioned by several researchers [34–36], these preferential interactions may not be the sole determining factors for chiral discrimination. Additional interactions, such as the steric interaction between the substituent group attached to the chiral centre of the chiral selectrand and the alkyl group attached to the chiral centre of the chiral selector, may also be involved to some extent in the chiral recognition process.

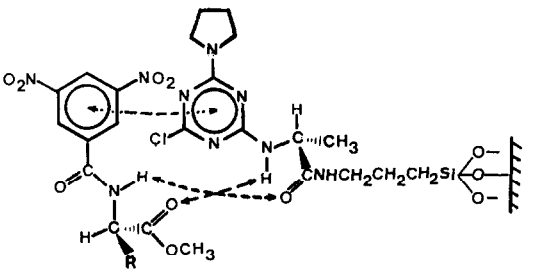
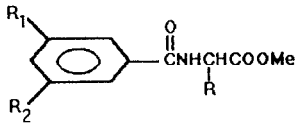


Fig. 3. Preferential interactions between methyl ester of an N-(3,5-dinitrobenzoyl)amino acid and the chiral stationary phase.

In order to establish whether the $\pi-\pi$ interaction involving the 3,5-dinitrobenzoyl group in the chiral selectrand and the pyrrolidinyl-substituted *s*-triazine ring in the chiral selector is essential for chiral recognition, we examined the effect of various π-acceptor substituents on the enantioseparation of methyl esters of benzoyl-derivatized amino acids so that the role played by the 3,5-dinitrobenzoyl group of the chiral selectrand in chiral recognition could be evaluated. Table 2 presents the chromatographic results of the dependence for various derivatized methionine methyl esters and those of valine methyl esters. According to Table 2, the replacement of a 3,5-dinitrobenzoyl group by a 3-nitrobenzoyl group or a benzoyl group greatly decreases the enantioselectivity. For instance, the α value decreased from 1.46 to 1.13 and 1.00 on replacement by a 3-nitrobenzoyl and a benzoyl group, respectively. This effect is simply due to the decrease in the $\pi-\pi$ interaction between the *s*-triazine ring of the chiral selector and the benzoyl group of the chiral selectrand. Our chromatographic data thus clearly indicate the necessity for the presence of a pyrrolidinyl-substituted *s*-triazine ring in the chiral selector and the importance of the interaction between π-acceptor and π-donor groups in the chiral discrimination process. This result is consistent with the findings of Pirkle and co-workers [11–13].

Table 2
Effect of different π-acceptor substituents on the enantioseparation of methylesters of benzoyl-derivatized amino acids



R_1	R_2	R = $CH_2CH_2SCH_3$			R = $CH(CH_3)_2$		
		k_1'	k_2'	α	k_1'	k_2'	α
NO_2	NO_2	5.02	7.35	1.46	2.38	3.67	1.54
H	NO_2	3.26	3.69	1.13	1.55	1.83	1.18
H	Cl	1.34	1.34	1.00	0.66	0.62	1.00
H	H	1.45	1.45	1.00	0.64	0.64	1.00

Eluent, 2-propanol–*n*-hexane (20:80, v/v); flow-rate, 1 ml/min.

The important role played by the ester group of the chiral analytes in the chiral discrimination has been demonstrated by Salvadori *et al.* [37], based on the fact that the replacement of an ester group with a cyano group greatly decreases the α value. We obtained additional evidence to support the important role of the ester group in the chiral recognition by comparing the chromatographic resolution of the enantiomers of 3,5-dinitrobenzoylamino acids with that of 3,5-dinitrobenzoylamine [38]. As the absence of the carbonyl group in 3,5-dinitrobenzoylamine excludes the formation of hydrogen bonding between the carbonyl group of the chiral selectrand and the secondary amino group of the chiral moiety of the chiral stationary phase, no chiral discrimination was achieved on this CSP for 3,5-dinitrobenzoylamines.

The role of the secondary amino group in the chiral analytes is seen by comparing the chromatographic results of the enantioseparation of esters of 3,5-dinitrobenzoyl amino acids possessing an acidic NH group with that of the ester of 3,5-dinitrobenzoylproline. The inability to achieve enantioseparation of the proline ester is probably due to the absence of an acidic NH group, because no hydrogen bonds could be formed between the chiral analyte and the chiral stationary phase. Although this hydrogen bonding is considered as the third preferential interaction, such an interaction may not be essential for the chiral discrimination, because no enantioseparation of amino acids was achieved on the chiral stationary phase derived from L-phenylglycyl- and pyrrolidinyl-disubstituted cyanuric chloride, despite the three major preferential interactions persisting between the CSP and the methyl ester of 3,5-dinitrobenzoylphenylalanine [38]. In other words, the results reveal that the enantiomers of an amino acid may be unable to be discriminated by these three preferential interactions alone. Instead, as was demonstrated in Table 1, steric repulsion between the substitutional group attached to the chiral centre of the chiral analyte and the substituent group attached to the chiral centre of the chiral stationary phase needs to be taken into consideration as a significant discrimination interaction in the chiral recognition process.

4. Conclusions

The chiral stationary phase derived from an L-alanyl- and pyrrolidinyl-disubstituted cyanuric chloride could provide excellent enantioselectivities for most of the racemates of amino acids tested, except proline. Although the π–π interaction and the two hydrogen bondings between chiral analytes and the chiral stationary phase are considered to be the three major preferential interactions between the chiral selector and chiral selectrands, the hydrogen bonding between the secondary amino group of the chiral

analytes and the carbonyl group of the chiral stationary phase may not be essential for the chiral discrimination. Instead, the steric interaction between the substituent group attached to the chiral centre of the chiral selectrands and the methyl group linked to the chiral centre of the chiral stationary phase should play a significant role in the chiral discrimination.

Acknowledgement

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