Chiral Recognition Models of Enantiomeric Separation on Cyclodextrin Chiral Stationary Phases

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The enantiomeric separation of several amino acid derivatives by reversed-phase liquid chromatography using two (R)- and (S)-naphthylethylcarbamate-β-cyclodextrin(NEC-β-CD) bonded stationary phases was studied to illustrate the chiral recognition model of the enantiomeric separation. The retention and enantioselectivity of the chiral separations with (R)- and (S)-NEC-β-CD bonded phases were compared with similar separations with the native β-CD stationary phases. Especially, the enantioselectivity and elution orders between the derivatized amino acid enantiomers are carefully examined. These results can be illustrated by the chiral recognition models involving inclusion complexation, π-π interation, and/or hydrophobic interation. Inclusion complexation and hydrophobic interation of the naphthyl group of the NEC moiety seem to be major chiral recognition components in the enantiomeric separation of 2,4-dinitrophenyl amino acids and dabsyl amino acids on (R)- and (S)-NEC-β-CD columns. For dansyl amino acids, only the inclusion complexation is the dominant factor. Three different chiral recognition models containing π-π interaction, inclusion complexation and hydrogen bonding were proposed for the separation of the 3,5-dinitrobenzoyl amino acid enantiomers, depending on the size and shape of amino acids.

Introduction

Cyclodextrins (CDs), which are cyclic oligosaccharides containing D(+)-glucopyranose units, have been used to develope a series of bonded chiral stationary phases. The functionalized CD bonded phases were introduced for enantiomeric high performance liquid chromatography (HPLC) separations in the normal-phase[1,2] as well as in the reversed -phase mode.[3,4] In order to illustrate the chiral recognition mechanism of enantiomeric separations, a number of empirical and theoretical studies have been done.[5~10] The formation of an inclusion complex seems to be a critical part of the chrial recognition and of separation process in the CD systems.[11~13] It is apparent that the size and geometry of a guest molecule compared with that of the cyclodextrin cavity is an important factor in inclusion complex formation. Inclusion complex formation is affected by the several different factors which include hydrophobic effect, hydrogen bonding, Van der Waals interations, release of high-energy water from the CD cavity and a change in ring strain upon complexation.[14] Since the CD molecules are themselves chiral, they can form a diastereomeric pair of inclusion complexes with each enantiomer of a racemate.

Consequently, the use of CDs in HPLC as a chiral stationary phase (CSP) bonded to solid support has been extensively studied. Enantiomeric resolution of a series of amino acids, barbituric dioxolane, phenylacetic acid derivatives, nicotine analogues and many other compounds were successfully carried out with β-CD CSPs under reversed phase conditions. Amino acids are one of the most important compounds which are found in all known living organisms. The enantiomeric separation of some free aromatic amino acids was only succeeded on a α-CD-CSPs[15] but not on the other CD columns. Most derivatized amino acids can be resolved on functionalized β-CD bonded CSPs.[4,13,16,17] The retention behavior and entioselectivity of the derivatized amino acids was dependent upon the kinds of the derivatization of amino acids and also upon the modification of the primary and secondary hydroxyl groups of CDs. Amino acids were usually derivatized with achiral aromatic compounds in order to form an inclusion complex with CDs. The substitution of the hydroxy groups in CDs with 1-naphthylethyl carbamate gives an additional stereogenic center, which can achieve a chiral recognition for the enantiomeric separation.

The goal of this paper is to obtain a better knowledge of the chiral recognition mechanism of the derivatized amino acids on two different naphthylethyl carbamate β-cyclodextrin bonded stationary phase, (S)-1(1-naphthyl)-ethyl carbamate [(S)NEC]-derivatized β-CD bonded phase column (SN) and (R)-1(1-naphthyl)-ethyl carbamate [(R)NEC]-derivatized β-CD bonde phase column (RN) in relation to native β-CD column. These RN and SN columns are able to form inclusion complex with the analyte by utilizing the CD cavity as well as the π-π interaction between the π-acidic portion of the analyte and the π-basic naphthyl portion of the columns. Primarily, we are interested in what the main chiral recognition for the enantiomeric separation of the derivatized amino acids is between the inclusion complex formation and/or the π-π interaction. Next, in the case of more than one aromatic group in the analyte it is critical that which one is fittable for the inclusion complex formation. The retention and enantioselectivity of the analyte on the two NEC-β-CD bonded CSPs will be determined. The elution orders between the enantiomers will be discussed by their chiral recognition model.

Experimental

Chemicals and Methods. Amino acids and other compounds were obtained from Sigma (St. Louis, MO, USA) or Aldrich (Milwaukee, WI, USA). HPLC-grade methanol (MeOH) and acetonitrile (MeCN) were purchased from Merck (Darmstadt, Germany). Water was deionized by passing distilled water through a ELGA purification system.

Free amino acids was dried and dansylated with dansyl (DNS) chloride according to the procedure described.[18,19]

Their dabsylation was also carried out by mixing 2.5×10^{-3} M each amino acids with 5×10^{-3} M dabsyl (DAB) chloride *via* the equal volume ratio.[20] In the 3,5-dinitrobenzoyl (DNB) derivatization, approximately 3-5 mg of amino acid were dissolved in 2 mL acetone. Next, 2-3 mg of 3,5-dinitrobenzoyl chloride derivatizing agent were added. The reaction solution was agitated in the heating bath at 60 ℃ for 10 min. The resulting solution was turned from dark brown to colorless and then was cooled and injected directly into the liquid chromatograph. In the 2,4-dinitrophenyl (DNP) derivatization of amino acids, the amino acid was dissolved in 1 mL of 2% sodium bicarbonate and mixed with 2 mL of ethanol containing 0.05 mL of 2,4-dinitrofluorobenzene. This mixture was shaken for 2 h in the dark at room temperature. The resulting solution was injected into the liquid chromatograph for the enantiomeric separation of the DNP-amino acids.

Apparatus. A liquid chromatographic system of Varian 5000LC with Rheodyne Injection valve Model 7125 was used as HPLC. A variable wavelength detector, Fluorichrom II was also used and interfaced with Varian 4290 integrator. The detection wavelength of DAB- and DNP-amino acids were set at 436 nm and 360 nm, respectively. The DNS- and DNB-amino acids both were detected at 254 nm. The pH of the mobile phase was adjusted by a DMS pH/ion meter of Model DP-215.

The three different 25 cm columns (4.6 mm I.D., 5 μm particle diameter) were obtained from Astec (Whippany, NJ, USA). The first column was a Cyclobond I column (BC) with β-CD molecules chemically bonded to silica gel with a five-atom non-nitrogen containing spaces. The other two columns were (S)-1(1-naphthyl)-ethyl carbamate [(S)NEC]-derivatized β-CD bonded phase column (SN) and (R)-1(1-naphthyl)-ethyl carbamate [(R)NEC]-derivatized β-CD bonded phase column (RN), which have one stereogenic center around the π-basic naphthyl group.

Results and Discussion

Inclusion complex formation of the analytes with CD is utilized in a liquid chromatographic system as a retention mechanism. Since the interior of the cavity of CDs is relatively hydrophobic, the capacity and separation factors of analytes are determined by hydrophobic interaction within cavity and also affected by the fittness of the analytes to the size of the CD cavity. Recently resolution of racemic derivatized amino acids on six CD stationary phases was systematically investigated.[4] It was concluded that the (R) NEC-β-CD column (RN) was the most widely useful derivatized cyclodextrin based CSP and the chiral recognition mechanism of this CSP involves inclusion complexation with β-CD cavity, π-π interaction with the naphthyl moiety, interactions with remaining chiral secondary alcohols at the CD mouth and steric hinderance. However, it was not sure whether the major factor of the chiral recognition mechanism for the separation of racemic derivatized amino acids is the inclusion complexation with the β-CD, the hydrophobic interaction of chiral NEC moiety, or their combination. It is natural that the substitution onto the β-CD column affect the retention and enantioselectivity of the racemic derivatized amino acids in a liquid chromatography. These can be speculated as follow. First, if the inclusion complex formation of the analytes with the CD is major chiral recognition mechanism without any effect of the NEC substitution group due to the steric effect of the bulky naphthyl moiety on the mouth of the CD cavity, their enantioselectivity will be decreased. Second, a new chiral recognition mechanism of the inclusion complexation including the interaction of the chiral NEC moiety can be created resulting no difference depending on the chirality of the NEC group. The elution orders of the enantiomers between use of RN and SN columns will be the same. Third, if only the π-π interaction of the chiral NEC moiety is the main factor for the chiral recognition mechanism of the liquid chromatographic separation without the inclusion complexation of the analytes with the β-CD cavity, the elution orders of the enantiomers will be reversed by replacing RN column with SN column.

Table 1. Capacity Factor (k') and Selectivity (α) of 2,4-Dinitrophenyl Amino Acids (DNP-AAs) Enantiomers on Several Cyclodextrin Chiral Stationary Phases

DNP-	BC		RN		SN		Mobile
AAs	k'	α	k'	α	k'	α	Phase
Val	4.49	0.98	5.98	0.90	6.16	0.97	A
	1.55	1.00	2.50	0.93	2.41	1.00	B
Leu	4.95	0.93	7.23	0.89	7.89	0.93	A
	1.62	1.00	3.02	0.92	2.98	0.95	B
Ile	4.93	0.95	7.57	0.89	8.12	0.97	A
	1.63	1.00	3.10	0.91	3.14	1.00	B
Phe	7.38	0.95	17.8	0.93	16.6	0.93	A
	1.80	1.00	4.71	1.00	4.29	1.00	B
Tyr	7.69	1.00	–	–	–	–	A
	1.12	1.00	–	–	9.78	1.00	B
Phg	6.90	1.00	15.4	1.00	14.9	1.00	A
	1.62	1.00	4.69	1.00	3.66	1.00	B

The capacity factors (k') stand for the first eluting enantiomers and the selectivity ($\alpha=k_D'/k_L'$). The columns are BC; Cyclobond I BETA. RN; Cyclobond I BETA-RN. SN; Cyclobond I BETA-SN. The mobile phases are A; MeOH-0.5% TEAA buffer (50 : 50) at pH 5.0. B; MeCN-0.5% TEAA buffer (40 : 60) at pH 5.0.

Separations of 2,4-Dinitrophenyl Amino Acids. There was a report on the enantiomeric separation of some DNP-amino acids only on β-CD bonded stationary phase, which discribed the resolution of 8 nonessential and 2 essential DNP-amino acids.[17] In this paper the optical resolutions of the racemic 6 different essential DNP-amino acids according to use of β-CD, (R)NEC-β-CD, and (S)NEC-β-CD CSP columns were compared. As shown in Table 1, it was found MeOH gave the better enantioselectivity than MeCN. We believe that the MeCN solvent of the low polarity may enter the β-CD and hinder the inclusion complex formation of the analytes compared with MeOH. The enantioselectivity of DNP-AAs on RN or SN column was greater than that on BC column. In principle, DNP-amino acids can penetrate β-CD cavity in only two orientations, either the *p*-nitro group of the DNP substituent first or the side chain of the amino acid first. The orientation of inserting the other on meta

Table 2. Capacity Factor (k') and Selectivity (α) of 3,5-Dinitrobenzoyl Amino Acids (DNB-AAs) Enantiomers on Several Cyclodextrin Chiral Stationary Phases

DNP-	BC		RN		SN		Mobile
AAs	k'	α	k'	α	k'	α	Phase
Leu	1.35	1.00	2.88	0.77	3.39	1.00	C
Ile	1.39	1.00	3.00	0.95	3.63	1.00	C
Met	1.84	1.00	3.33	0.61	3.32	0.93	C
Tyr	1.82	1.00	3.73	0.94	3.70	1.17	C
Phg	1.74	1.00	4.94	0.70	4.71	1.57	C
Phe	3.40	0.89	7.02	1.10	4.15	1.06	D
Trp	3.29	0.97	7.58	1.09	2.66	1.11	D
Ala	1.33	1.00	2.94	0.97	1.96	1.05	D
Asp	2.52	1.00	6.91	1.00	3.86	1.06	D
Thr	1.28	1.00	2.48	1.07	2.66	1.11	D

The columns are the same as in Table 1. The mobile phases are C; MeCN-0.7% TEAA buffer (50 : 50) at pH 5.8. D; MeCN-0.5% TEAA buffer (30 : 70) for BC column and MeCN-0.5% TEAA buffer (50 : 50) for RN and SN columns at pH 4.

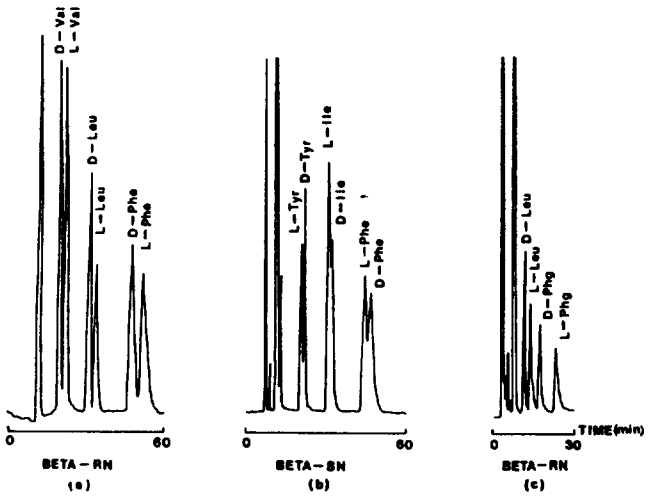

Figure 1. Chromatograms of DNP-(a), DNS-(b), DNB-Amino Acids(c) enantiomers on derivatized β-Cyclodextrin columns. Mobile Phase condition a) 50% MeOH : 50% Buffer Solution (0.5 % TEA, pH 5.5). b) 20% MeCN : 80% Buffer Solution (0.7% TEA, pH 4.5). c) 50% MeOH : 50% Buffer Solution (0.7% TEA, pH 5.8) Flow Rate 1.0 mL/min.

position of the DNP substituent is impossible for them to fit onto the cavity. It was reported that the depth and the tilt of the aromatic ring in the cavity are significantly different for the D and L enantiomers in the inclusion complexation of DNP-amino acids. Li and Purdy[12] suggested *via* NMR study that the tilt angle and insertion depth of DNP-amino acids in the inclusion complex formation of DNP-amino acids with β-CD were different. Therefore they concluded that two or even more inclusion complexes rather than 1 : 1 stoichiometry can be associated between DNP-amino acids and free CD molecules. However in liquid chromatographic systems having solid CD bonded phases such as β-CD, RN or SN column, it is not reasonable to think the existence of the inclusion complex of other than 1 : 1 stoichiometry because the CD cavity is not populated in the columns and also not close enough to form the inclusion complex of two CDs bonded to the solid support with the analytes. So it should be a 1 : 1 inclusion complex with the different tilt angle and insertion depth. As shown in Table 1, the longer retention times were observed on the less polar RN and SN stationary phases due to the more hydrophobic interaction and the enantioselectivity is enhanced a little by substituting the NEC group to the CD column. However, the elution orders of DNP-AAs were not altered by using three different columns. These facts indicate that the chiral recognition mechanism of the DNP-AAs is not different on these three cyclodextrin-based columns with minor modification of the inclusion complex formation.

Separation of 3,5-Dinitrobenzoyl Amino Acids. The DNB derivatization adds a π-acid group to the amino group of amino acids. The DNB group has greater π-acidity than the DNP group which may favor CD inclusion. The DNB group tends less to form the inclusion complex with the CDs which is more favorable to π-basic group than π-acidic groups in aqueous system. Therefore, as shown in Table 2, for the native CD bonded column a few compounds such as Phe and Trp can be enantiomerically separated. These compounds have an aromatic group which may be filtable to the inclusion complex formation.[4] From the results it could be concluded that the DNB group was not able to penetrate the β-CD cavity to form inclusion complex. However, it could be thought that the aromatic side chains of DNB-Phe and DNB-Trp can penetrate into the β-CD cavity to form inclusion complex with difference in stability. But those of DNB-Tyr and DNB-Phg are not easy to form inclusion complex because the hydrogen bonding of the hydroxyl group of the Tyr aromatic side chain with the secondary hydroxyls of CD cavity is possible and because the size of the phenyl side chain of DNB-Phg is not adequate to form inclusion complex between the enantiomers with difference in chiral recognition. For the enantiomeric separation of the 3,5-dinitrobenzoyl amino acids it can be classified as three different elution types. First, phenylalanine and tryptophan having the side chain of aromatic group showed a chiral resolution on all the three CD columns. The better resolution on the NEC-columns was observed than on β-CD column. The elution orders of the enantiomers on the β-CD column were reversed when the (R)- or (S)-NEC-β-CD columns were used. However, no difference in the elution orders of the enantiomers between RN and SN columns depending on the chirality of the NEC moiety was observed. It indicates that the NEC group of the columns is important for the chiral separation but not dominant factor in the chiral recognition. Therefore, we can see that the inclusion complexation of the aromatic side chains with β-CD cavity as well as π-π interaction of DNB-amino acids with the naphthylethyl group of the NEC moiety is involved for the enantiomeric separation. A typical chromatogram for the result was shown in Figure 1. Second, the chiral recognition of DNB-Met and DNB-Thr were not observed on the β-CD column but on the RN and SN columns. The elution order of amino acid enantiomers on these columns was the same but that between DNB-Met and DNB-Thr different. It was reasonable

Table 3. Capacity Factor (k') and Selectivity (α) of Dansyl Amino Acids (DNS-AAs) Enantiomers on Several Cyclodextrin Chiral Stationary Phases

DNS-AAs	BC		RN		SN	
	k'	α	k'	α	k'	α
Val	0.66	1.18	4.50	1.06	1.48	1.09
Leu	0.65	1.18	5.38	1.00	1.66	1.00
Ser	0.79	1.11	2.48	1.04	1.01	1.06
Thr	0.82	1.20	2.96	1.06	1.09	1.08
Phe	0.64	1.14	7.01	1.09	1.94	1.08
Met	0.54	1.22	4.69	1.00	1.46	1.00

The mobile phase for BC column is 35% MeCN and 65% buffer solution (1.0% TEAA) at pH 4.5 while that for RN and SN columns is 50% MeCN and 50% buffer solution (1.0% TEAA) at pH 4.5. The columns used are the same as in Table 1.

to think that the π-π interaction of the DNB-amino acids with the naphthylethyl group of the NEC-β-CD columns be also involved in the chiral separation. The side chain of methionine and threonine containing sulfur atom or hydroxyl have hydrogen bonding ability. These compounds can form not inclusion complexation but hydrogen bonding with the secondary hydroxyl group of the CD moiety. So the chirality of the NEC moiety of the columns is not main factor for the enantiomeric separation. It is not clear that the elution orders between the enantiomers of DNB-Met and DNB-Thr were opposite. The hydrogen bonding of these molecules can be sepasrated to occur differently to the C-2 or C-3 hydroxyl groups of the β-CD hydroxyls, respectively, It is well known that the secondary hydroxyl group (C-2 and C-3) are fixed in space with all of the C-2 hydroxyl groups pointed in a clockwise direction and all of the C-3 hydroxyl groups pointed in a counter-clockwise direction.[21] Third, all the order 3,5-dinitrobenzoyl amino acids with no aromatic side chains including DNB-Phg and DNB-Tyr showed no enantiomeric resolution on the native CD column but some resolution on the RN and/or SN columns. The elution orders of the DNB-AAs enantiomers on the RN column were reversed when the SN column was used except DNB-Leu and DNB-Ile. We believe that these enantiomers were not resolved on the SN column due to low coverage of the NEC moiety. It indicates that the naphthylethyl group of the NEC moiety is important to form π-π interaction with the DNB-amino acids so the chirality of the NEC is critical in the chiral recognition. This result descrives the π-π interaction as a dominant chiral recognition. However, the β-CD moiety of the RN or SN column does not seem to affect the chiral recognition.

Separation of Dansyl Amino Acids. Amino acid enantiomers with the dansyl group having a bulky naphthyl group were relatively resolved well on the native β-CD column. But the enantiomeric resolutions of DNS-AAs were decreased by replacing the β-CD column with RN or SN column as shown in Table 3. The elution orders of the DNS-amino acid enantiomers on the three different CD chiral columns were the same. The dansyl derivatives, with a naphthyl group, seem to fit well in the β-CD cavity.[4] These results

Table 4. Capacity Factor (k') and Selectivity (α) of Dabsyl Amino Acids (DAB-AAs) Enantiomers on Several Cyclodextrin Chiral Stationary Phases

DAB-	BC		RN		SN		Mobile
AAs	k'	α	k'	α	k'	α	Phase
Val	4.69	0.98	–	–	13.0	0.83	E
Phe	4.74	1.00	13.0	0.97	20.5	1.15	E
Glu	19.0	1.00	9.04	0.95	20.1	0.98	E
Met	3.94	0.98	9.18	0.96	13.9	0.84	E
Leu	4.32	1.01	–	–	18.1	0.79	E
Ile	4.52	1.00	–	–	22.9	0.97	E
Ser	2.63	1.05	1.43	0.84	0.80	0.94	F
Trp	2.41	0.90	1.63	0.90	2.68	0.94	F
Thr	3.08	1.00	1.86	0.81	1.03	0.93	F
Ala	2.58	1.00	1.82	0.88	0.96	0.94	F

The mobile phases are E; MeCN-0.5% TEAA buffer (40 : 60) at pH 4.5. F; MeCN-1% TEAA buffer (40 : 60) for BC column and MeCN-1% TEAA buffer (65 : 35) for RN and SN columns at pH 6.0.

show that the main chiral mechanism of DNS-AAs on these columns is inclusion complexation and the NEC moiety of RN or SN column decreases the chiral recognition due to the steric effect against the inclusion complexation. So the bulky NEC group of the columns may hinder for the dansyl group of DNS-AAs to penetrate the cavity of CDs to form a stable inclusion complex.

Separation of Dabsyl Amino Acids. The dabsyl deraivative is more π-basic than the DNS derivative. It was chosen for its long and rigid azobenzene backbone which may favor CD inclusion. Table 4 list the capacity and enantioselectivity factors of the dabsyl amino acids on the three CD columns. The results show that as a general rule, the enantioselectivity of DAB-AAs on the RN and SN columns is greater than that on the β-CD column. The elution orders of the DAB-AAs enantiomers were the same through the three different CD columns except DAB-Phe and DAB-Ser. The enantiomer elution order of all dabsyl amino acids on β-CD and NEC-β-CD bonded stationary phases was D before L enantiomer, while a reverse elution order for dansyl amino acids was observed. These result of the opposite elution order can be illustrated as a tight fittness of the inclusion complexation to the cavity of the cyclodextrin. The bulky dansyl group fits well on the cyclodextrin cavity but the long dabsyl group fits loose. The DAB-AAs may form a hydrogen bonding of *p*-dimethylamine of their long azobenzene backbone with the primary hydroxyls of the β-CD column so their chiral interactions seem to be different from those of DNS-AAs. Therefore, the DAB-AAs, which has its long and rigid azobenzene backbone, may favor CD inclusion complexation containing a hydrophobic interaction of the nonpolar side chains of DAB-AAs with the naphthyl group of the NEC moiety. For the enantiomeric separation of DNS-amino acids, the chirality of the CDs in inclusion complexation takes a major role in the chiral recognition model. On the other hand, for the enantiomeric separation of DAB-amino acids, both the CD cavity and the NEC group are involved in the

chiral recognition model. It is not clear that the exceptional retention order of DAB-Phe on the SN column was resulted but it can be assumed that the phenyl ring of the Phe side chain rather than the dabsyl group may penetrate the CD cavity to form the inclusion complex on this column. The futher study on this curious retention behavior is required to understand the specific chiral recognition for DAB-Phe.

Conclusion

The (R)- or (S)-NEC-β-CD column, which was β-CD column bonded with chiral naphthylethyl isocyanate, was used for the enantiomeric resolution of four different derivatives of amino acids (*i.e.*, DNP-, DNB-, DNS-, and DAB-Amino acids) to study the chiral recognition model based on that of the native β-CD column. The three substituted groups (DNP, DNS, and DAB) of the four derivatized amino acids take an important role in the inclusion complexation but the DNB group of DNB-AAs is too bulky to form inclusion complex fittable for β-CD cavity. It could be concluded that the chiral recognition mechanism of the DNP-AAs and DAB-AAs was based on the inclusion complexation of the substituted groups containing the hydrophobic interaction of the nonpolar amino acid side chains with the naphthyl group of the NEC moiety. Similarly, that of the DNS-AAs was also based on the inclusion compexation of the DNS group with β-CD cavity, but decreasing the chiral recognition due to the steric effect of the column NEC moiety. On the other hand, it can be speculated that there are three different chiral recognition mechanisms for the enantiomeric resolution of DNB-amino acids on RN or SN column. First, the π-π interaction of DNB-amino acids with the naphthyl group of the NEC moiety involving the inclusion compexation of the aromatic side chains for aromatic amino acids was involved. Second, for DNB-Met and DNB-Thr, the π-π interction with the NEC moiety plus the hydrogen bonding with the secondary hyroxyl group of the CD moiety was found. Third, for most of the DNB-AAs, only the π-π interaction of DNB group with the chiral NEC moiety was a dominant factor in the chiral recognition mechanism.

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Reference

1. Armstrong, D. W.; Stalcup, A. M.; Hilton, M. L.; Duncan, J. D.; Faulkner, J. R; Chang, S. C. *Anal. Chem.* **1990**, *62*, 1610.
2. Stalcup, A. M.; Chang, S. C.; Armstrong, D. W. *J. Chromatogr.* **1991**, *540*, 113.
3. Armstrong, D. W.; Chang, S. C.; Lee, S. H. *J. Chromatogr.* **1991**, *539*, 83.
4. Lee, S. H.; Berthod, A.; Armstrong, D. W. *J. Chromatogr.* **1992**, *603*, 83.
5. Berthod, A.; Chang, S. C.; Armstrong, D. W. *Anal. Chem.* **1992**, *64*, 395.
6. Sabio, M.; Topiol, S. *Chirality* **1991**, *3*, 56.
7. Pirkle, W. H.; Pochapsky, T. C. *J. Chromatogr.* **1986**, *396*, 175.
8. Lipkowitz, K.; Landwere, J. M.; Darden, T. *Anal. Chem.* **1986**, *58*, 1611.
9. Topiol, S.; Sabio, M.; Moroz, J.; Caldwell, W. B. *J. Am. Chem. Soc.* **1988**, *110*, 8367.
10. Boehm, R. E.; Martire, D. E.; Armstrong, D. W. *Anal. Chem.* **1988**, *60*, 522.
11. Ward, T. J.; Armstrong, D. W. *J. Liq. Chromatogr.* **1986**, *9*, 407.
12. Li, S.; Purdy, W. C. *Anal. Chem.* **1992**, *64*, 1405.
13. Fujimura, K.; Suzuki, S.; Hayashi, K.; Masude, S. *Anal. Chem. 62*, 2198.
14. Armstrong, D. W.; Demond, W. *J. Chromatogr. Sci.* **1984**, *22*, 411.
15. Armstrong, D. W.; Yang, X.; Han, S. H.; Menges, R. A. *Anal. Chem.* **1987**, *59*, 2594.
16. Hinze, W. L.; Riehl, T. E.: Armstrong, D. W.; Demond, W.; Alak, A.; Ward, T. J. *Anal. Chem.* **1985**, *57*, 237.
17. Li, S.; Purdy, W. *J. Chromatogr.* **1991**, *543*, 105.
18. Lee, S. H.; Oh, T. S.; Bak, S. H. *Bull. Korean Chem. Soc.* **1989**, *10*, 491.
19. Lee, S. H.; Oh, T. S.; Park, B. J. *J. Korean Chem. Soc.* **1990**, *34*, 76.
20. Lee, S. H.; Oh, T. S.; Lee, Y. C. *Bull. Korean Chem. Soc.* **1990**, *11*, 411.
21. Menges, R. A.; Armstrong, D. W. In *Chral Separations by Liquid Chromatography*; Ahuza, S., Ed; CIBA-GEIGY Co. Washington D. C. 1990, Chapter 4.