Arch. Pharm. Res. **12**(1), 58-61 (1989)

Chiral Recognition in Gas chromatographic Resolution of Amino Acid Enantiomers

^{1}H and ^{13}C Nuclear magnetic resonance studies of hydrogen bonding in diamide chiral stationary phases

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(Received February 12, 1989)

Abstract □ Studies of selectivity of hydrogen bond formation in chiral solute-solvent systems have been performed by ^{1}H and ^{13}C nuclear magnetic resonance techniques. These data are correlated with the results of gas chromatographic investigations of the same systems. Interactions between the optically active solvent(N-(N-benzoyl-L-amino acid)-anilide) and optically active solute(N-trifluoroacetyl-L-alanyl isopropyl ester) were examined. NMR evidence indicated that hydrogen bonding interaction occurred between two N-H portion and on peptidyl carbonyl portion in stationary phase and solute molecule on three points. The association constants of solvent-solute interaction were calculated and the structure of the diastereomeric association complex between N-(N-benzoyl-L-valyl)-anilide and N-TFA-L-alanyl isopropyl ester was proposed.

In recent years, researches about the resolution of amino acid enantiomers on diamide chiral stationary phase were carried out in our laboratory.[1-6] The separation of enantiomer by chiral stationary phase can be explained by "three point interaction".[7] That is, there should be at least three interaction points between the stationary phase and one of the enantiomers for the chiral stationary phase to recognize chirality. The interactions concerned with chiral recognition include hydrogen bonding, electron movement, steric hindrance, hydrophilicity, or hydrophobicity.

In diamide chiral stationary phase, Lochmüller *et al.* explained that D-valine eluted earlier than L-valine on dipeptide stationary phase due to the difference of the stability of hydrogen bonding.[8] Fig. 1 shows the interaction model Lochmüller suggested. In this model, L-enantiomer forms stable hydrogen bond with stationary phase, but D-enantiomer does not, so D-enantiomer elutes earlier. The authors suggested diamide stationary phase synthesized from L-amino acid as chiral stationary phase for the separation of amino acid enantiomers.[5-6] The surface of chiral stationary phase offers chiral environment and interacts with a pair of enantiomer. The enantiomers are separated by the difference of stability of the temporary diastereomeric complex.

To examine the hydrogen bonding interaction between stationary phase and analyte, the interpretation of the chemical shift from NMR spectrum is simple and powerful means.[9] The change of density of the electron around the nucleus of proton caused by hydrogen bonding induces chemical shift. From the chemical shift, information about the environment of the proton is obtained. The authors tried to confirm the separation mechanism by this NMR technique.

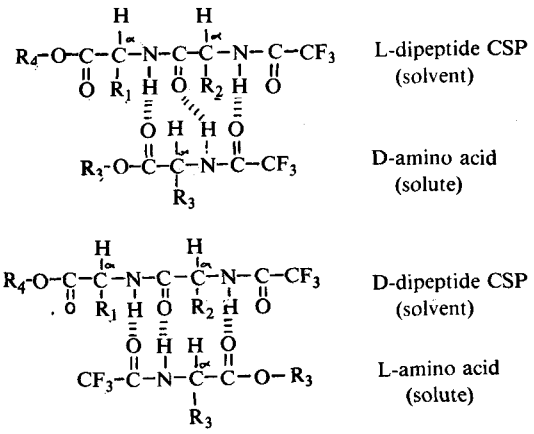

Fig. 1. Interaction of N-TFA-dipeptide ester (solvent) with D- and L- N-TFA-amino acid esters (solute). From C.H. Lochmüller and R.W. Souter, *J. Chromatogr.* **113**, 283 (1975).

EXPERIMENTAL METHODS

Materials

L-valine, L-isoleucine, L-leucine, L-phenylalanine, L-alanine, and diethylphosphocyanate (DEPC) were purchased from Sigma Chemical company. Trifluoroacetic anhydride (for GC analysis), deuterochloroform and tetramethylsilane (TMS) were purchased from Pierce Chem. Benzoyl chloride, aniline, triethylamine, ethyl acetate, methylene chloride of extra pure grade were used.

Methods

As for chiral stationary phase (solvent), N-(N-benzoyl-L-valine)-anilide, N-(N-benzoyl-L-isoleucine)-anilide, N-(N-benzoyl-L-leucine)-anilide, N-(N-benzoyl-L-phenylalanine)-anilide were synthesized, and as for analyte (solute), N-trifluoroacetyl-L-alanyl-isopropyl ester, were synthesized by the methods previous thesis described.[6] Each solution of 0, 4, 8, 12, 16, 20 mM N-trifluoroacetyl-L-alanyl-isopropyl ester in 100 mM N-(N-benzoyl-L-amino acid)-anilide $CDCl_3$ solution was prepared. ^1H and ^{13}C NMR spectra were recorded on a Varian 80 MHz FT-NMR spectrometer. Chemical shifts were read relative to the resonance of internal tetramethylsilane in each case.

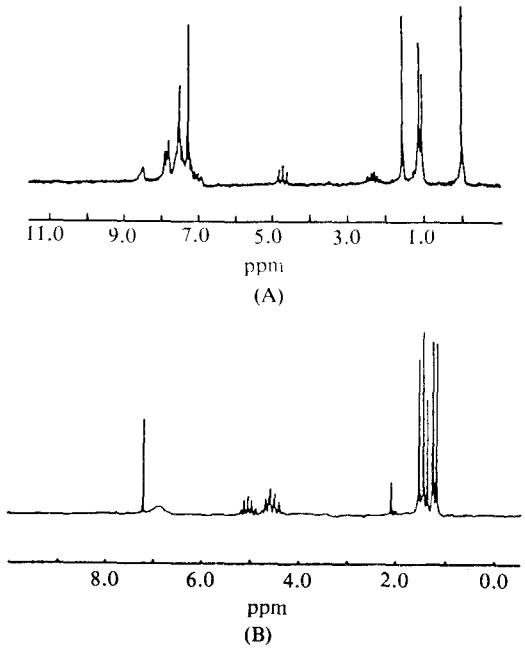

Fig. 2. NMR spectra of N-(N-benzoyl-L-valyl)-anilide (A) and N-trifluoroacetyl-L-alanine isopropyl ester (B) in deuterochloroform.

RESULTS AND DISCUSSION

When the electron density of the prton participating in hydrogen bond lowers, the shielding effect by the electron lowers, too, and proton peak moves to lower field. If the proton is participating in hydrogen bonding, the chemical shift of the proton changes as the concentration of solute changes. From the induced shift, the association constant of hydrogen bond between the stationary phase and the analyte can be calculated.

Fig. 2 is the NMR spectrum of N-trifluoroacetyl-L-alanyl-isopropyl ester in $CDCl_3$ used as solute and Fig. 3 is the NMR spectra of 100 mM N-(N-benzoyl-L-valyl)-anilide in $CDCl_3$ with the concentration of solute increasing. The peptidyl NH proton peak near 7ppm moved to lower field as the concentration of the analyte increased, and supe-

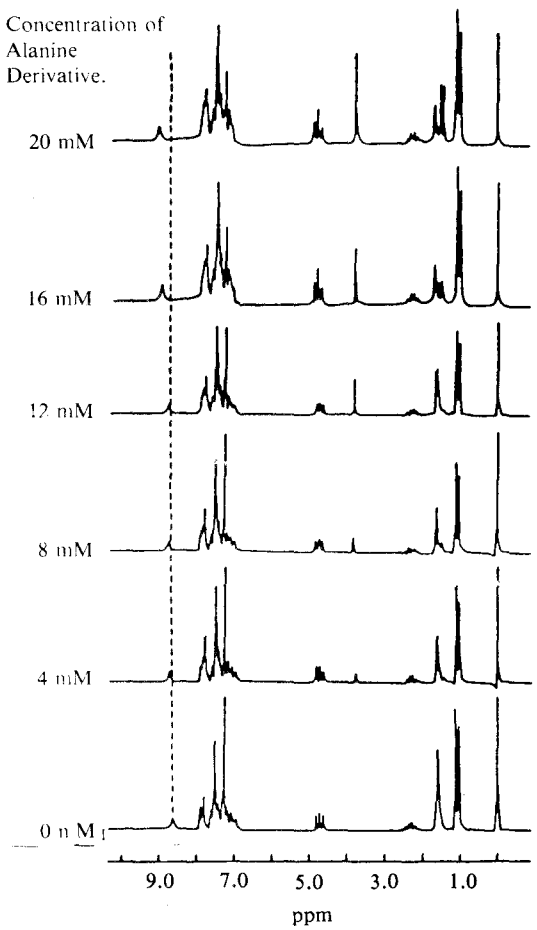

Fig. 3. Effects of the concentration of N-TFA-L-Ala-isopropyl ester on the spectra of N-(N-benzoyl-L-valyl)-anilide in deterochloroform.

rimposed to aromatic proton peak. The amide NH proton in the range of 8.0-9.0ppm also moved to lower field as the concentration of the analyte increased. It means that of the analyte increased. It means that these two NH protons are participating in hydrogen bond. From Hanna's equation,[10] induced shift of proton and the association constant is related as following equation:

$$\frac{1}{\delta_{obs}} = \frac{1}{K_c(\delta_c - \delta_a)} \cdot \frac{1}{C_b} + \frac{1}{(\delta_c - \delta_a)}$$

$\delta_{obs} = \delta_{obs} - \delta_a$

δ_a : chemical shift of uncomplexed form

δ_c ; chemical shift of given conc. of solute

C_b; conc. of solute

K_c; association constant

According to this equation, there is linearity between $1/C_b$ and $1/\delta_{obs}$ when association complex is formed from stationary and the analyte. Fig. 4 showed the linearity plotting of the inverse of the concentration of the analyte versus the inverse of the induced shift of the chiral stationary phase. The association constant can be calculated by least square method. Table I showed the association constant of the diastereomeric complex of each chiral stationary phase and the solute. The order of the association constant is correspondent with the elution order in chromatographic system.

To examine the proton acceptor group, CMR spectra of N-(N-benzoyl-L-valyl)-anilide and the mixture solution of NBVA and the analyte was measured. The result is in Table II. The peptidyl carbonyl carbon had no change in chemical shift after the addition of analyte, but amide carbonyl

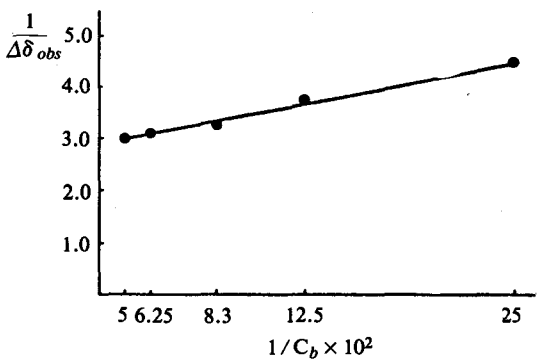

Fig. 4. Plot of the inverse of the concentration of N-TFA-L-Ala-isopropyl ester *versus* the inverse of the induced shift of N-(N-benzoyl-L-valyl)-anilide amide NH proton in deuterochloroform, keeping the concentration of N-(N-benzoyl-L-valyl)-anilide constant at 100 mM.

Table I. Effects of the concentrations of N-TFA-L-alanine isopropyl ester on the spectra of N-(N-benzoyl-L-amino acid)-anilide amide NH proton in deuterochloroform, keeping the concentration of N-(N-benzoyl-amino acid)-anilide constant at 100mM

Conc. of N-TFA-L-ala isopropyl ester (mM)	Chemical shift of N-(N-benzoyl-L-amino acid)-anilide NH proton (ppm)			
	valyl	isoleucyl	leucyl	phenyl-alanyl
0	8.53	8.23	8.60	9.00
4	8.75	8.46	8.85	9.12
8	8.80	8.49	8.89	9.16
12	8.83	8.52	9.01	9.18
16	8.85	8.53	9.05	9.20
20	8.86	8.54	9.06	9.21
Kc	0.328	0.541	0.451	0.232
r	0.995	0.985	0.981	0.997

Table II. Assignments of the ^{13}C magnetic resonance of carbonyl carbon of N-(N-benzoyl-L-valyl)-anilide in deutereochloroform and the induced shift on the addition of N-trifluoroacetyl-L-alanine isopropyl ester

Assignment	resonance position, ppm	induced shift, ppm
amide carbonyl	170.8	0.60
peptidyl carbonyl	168.0	0.01

carbon peak shifted to lower field by 0.6 ppm. From the result, it was sure that the amide carbonyl group was participating in hydrogen bonding as the proton acceptor.

Fig. 5 shows the interaction model of diastereomeric complex of N-(N-benzoyl-L-valyl)-anilide and N-TFA-L-alanyl-isopropyl ester. The aromatic rings at both end of the stationary phase arrange parallel on the surface of the parking material. The conformation resembled the pleated structure of β-keratin permitting the formation of the maximum number of hydrogen bonds. The methyl group of the solute fills the space between two isopropyl groups of stationary phase, and the structure is stabilized by van der Waals forces. The space-filling isopropyl and methyl groups acted as guides, keeping the diastereomeric association complexes "on track". As it is impossible for the D-enantiomer of the amino acid derivative to arrange to form the "track", the association complex of D-en-

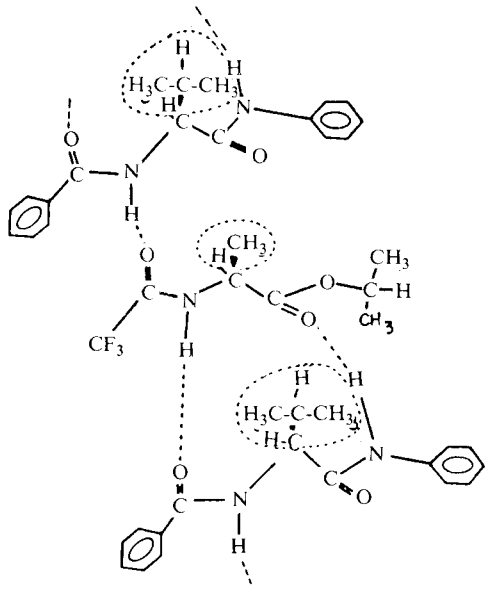

Fig. 5. Structure of the diastereoisomeric association complex between N-(N-benzoyl-L-valyl)-anilide and N-trifluoroacetyl-L-alanine isopropyl ester stabilized with "track" made by isopropyl and methyl groups.

antiomer is unstable. By this mechanism, the D-enantiomer was thrown "off track" and eluted earlier.

CONCLUSIONS

Hydrogen bond formation of diamide chiral solvent-solute system was checked by NMR technique. The association constants of hydrogen bonding were calculated. For the mechanism of chiral recognition, the analyte fills the "track", and the association complex is stabilized by the van der Waals force and so eluted earlier. The structure of diastereomeric association complex is shown.

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