Journal of Chromatography, 349 (1985) 143–154
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMSYMP. 668

CHIRAL RECOGNITION MECHANISMS IN THE ENANTIOSELECTIVITY OF CHIRAL HYDROGEN BONDING ADDITIVES IN LIQUID–SOLID CHROMATOGRAPHY

AKIRA DOBASHI and SHOJI HARA*
Tokyo College of Pharmacy, 1432-1 Horinouchi, Hachioji, Tokyo 192-03 (Japan)

SUMMARY

The addition of a chiral hydrogen bonding additive, derived from N-acetyl-L-valine, to the non-aqueous mobile phase in silica gel liquid chromatography permits the chiral recognition of D- and L-amino acid derivatives as N-acetyl-O-*tert.*-butyl esters. Chiral N-acetyl-L-valine-*tert.*-butylamide is firmly adsorbed on the silica gel surface and forms the chiral hydrogen-bonded phase. The recognition process by which enantioselectivity is attained occurs predominantly in this *de facto* stationary phase. The main driving force for the recognition may be attributed to steric effects, exerted by the silica gel surface in adsorbing the solute–additive associates, rather than to the stability difference between the diastereomeric hydrogen-bonded associates as observable in solution.

In contrast, the recognition process of chiral N-acetyl-L-valine-*tert.*-butyl ester occurs predominantly in the bulk of the mobile phase. This additive, which shows retention on the silica gel similar to that of the solutes to be resolved, has little effect on recognition in the stationary phase process. The enantioselectivity should therefore be ascribed to stability differences between the diastereomeric hydrogen-bonded associates in the mobile phase.

INTRODUCTION

This study is a continuation of our earlier work which showed that the addition of chiral hydrogen bonding additives (CHBA) to the non-aqueous mobile phase in liquid–solid chromatography permits the recognition of molecular chiralities. This is a unique recognition technique in which CHBA are regarded as "hydrogen-bonding solvents", which induce transient diastereomeric hydrogen-bond solvation with solute enantiomers and serve as a stronger solvent component to aid their separate elution. Up to now, versatility in chiral recognition with the use of CHBA has been known only in part for two CHBA: chiral N-acetyl-L-valine-*tert.*-butylamide (CHBA 1)[1–3] and (*R,R*)-diisopropyl tartramide[4,5]. The former is capable of resolving amino acid enantiomers as N-acetyl-O-*tert.*-butyl ester derivatives in silica gel liquid chromatography[1,2]. On the basis of its excellent selectivity, the scope of this method was extended to the highly sensitive resolution of complex mixtures of amino acid deriv-

0021-9673/85/$03.30

atives, such as N-(4-nitrobenzoyl)-O-isopropyl esters[3]. The explanation of the origin of resolution of the enantiomers has yet to be solved.

To assist in the solution of this problem, the following examinations were carried out: (1) determination of the amount of CHBA 1 adsorbed on a silica gel surface, (2) immobilization of the CHBA 1 structure on the surface of the gel and (3) resolution by use of an alternative L-valine additive, subjected to the same derivatization of the N-acetyl-O-*tert.*-butyl ester as that of the amino acid enantiomers to be resolved. This paper discusses the chiral recognition mechanisms responsible for the enantioselectivity of CHBA on the basis of the separation of D- and L-amino acid derivatives.

EXPERIMENTAL

Isocratic chromatography was performed with two liquid chromatographic systems. One was a liquid chromatograph with a micro-bore column, as used previously[3] but without a sample injector; a Rheodyne Model 7413 injector with a 0.5-μl loop was used instead. The other was also a laboratory-built system, equipped with a Merck Hibar pre-packed column (25 × 0.4 cm I.D.)[2]. This column contained 2.0 g of LiChrosorb Si 60 (5 μm) silica gel. For detection, UV absorption at either 265 or 254 nm was used: at 265 nm for the chromatographic experiments with CHBA 1 owing to transparency of the additive, and at 254 nm with N-acetyl-L-valine *tert.*-butyl ester (CHBA 2). The chromatographic columns were maintained at a constant temperature of 20°C.

Two dimensions of micro-bore columns were used. One column, slurry packed with silica gel [Spherosil XOA-600 (5 μm); Prolabo, Paris], was 25 × 0.1 cm I.D. and the other, packed with aminopropyl silica [Nucleosil-5NH_2 (5 μm); Macherey, Nagel & Co., Düren, F.R.G.], was 50 × 0.1 cm I.D. The amino packing contained 0.84 mmole of amino groups per gram, as calculated from the mean nitrogen contents obtained by three elemental analyses of this material (found: C, 3.81; H, 1.04; N, 1.17%).

The columns were packed by a high-pressure slurry technique according to the following procedure. About 600 mg of the packing, dried over P_2O_5 under reduced pressure, was suspended in 9 ml of methanol–2-propanol–carbon tetrachloride (2:3:11, v/v) with sonication. The homogenized mixture was poured into the slurry reservoir (1.0 cm I.D., inner volume 10 ml) and forced from the reservoir into the column using chloroform at 10 000 p.s.i. The column was then equilibrated with the chromatographic solvent, consisting of ethyl acetate *n*-hexane (20:80, v/v), to determine the number of theoretical plates of the column. The silica column had 7700 plates per 25 cm and the aminopropyl silica column 12 000 plates per 50 cm. These values were obtained using di-*n*-butyl phthalate as the solute at a solvent flow-rate of 60 μl/min.

All solvents used for chromatography were distilled prior to use.

Measurement of the amount of CHBA 1 adsorbed on the silica gel surface

The Merck Hibar column, with its bottom connected to a variable-wavelength UV detector (Jasco UVIDEC-100-II; Japan Spectroscopic, Tokyo, Japan), was immersed in a constant-temperature bath (Yamato Model BL-21) at 20°C. The column

was washed successively with 200 ml of ethyl acetate and chloroform, then equilibrated in chloroform–*n*-hexane (40:60, v/v). The hold-up volume of the column, attached to the liquid chromatograph, was determined by injecting benzene into the column. The above mixture, containing 15 m*M* of CHBA 1, was made to pass through the column, and the eluent from the column was collected until the silica gel column attained equilibrium with the mobile phase solvent. Equilibrium was confirmed by constant UV absorption by CHBA 1, monitored at 230 nm, and constant retention of the solute enantiomers of the racemic N-acetyl-N^{ind}-*tert.*-butyltryptophan *tert.*-butyl ester after three injections. After the volume of the combined eluents had been precisely measured, the solvent was removed under reduced pressure and the residual additive was weighed. The amount of CHBA 1 adsorbed on the silica gel surface was calculated as the loss of the additive in the mobile phase solvent corresponding to the particular volume of eluent from which the hold-up volume, determined prior to equilibration, had been removed. By this procedure, 196.06 mg of the CHBA 1 were found to be held on 2.0 g of the silica gel in the column.

The amount of CHBA 1 in the mobile phase bulk was considered to correspond to that in the alternative hold-up volume, as also determined by using benzene, following attainment of equilibrium of the additive. The observed amount was 9.64 mg of CHBA 1 in 3 ml of the hold-up volume.

Preparation of chiral acids and procedure for in situ modification of packed amino columns

The chiral graft glutaryl-L-valine-*tert.*-butylamide monocarboxylic acid was prepared by the following procedure. 4-Benzyloxycarbonylbutanoyl N-4-hydroxysuccinimide ester, obtained by dicyclohexylcarbodiimide coupling between glutaric acid monobenzyl ester and N-hydroxysuccinimide, and L-valine-*tert.*-butylamide hydrochloride [subliming at *ca.* 148°C; $[\alpha]_D^{23} = +45.6°$ ($c = 1.01$, methanol)] were condensed in the presence of triethylamine. The benzyl ester obtained was hydrogenated to afford the desired material. Recrystallization from ethyl acetate gave pure material of m.p. 143–147°C; ^{1}H NMR (C^2HCl_3), δ 0.93 (d, 3H, $J = 6.6$ Hz), 0.94 (d, 3H, $J = 6.6$ Hz), 1.35 (s, 9H), 1.79–2.09 (m, 3H), 2.23–2.54 (m, 4H), 4.01–4.10 (dd, 1H), 5.99 (brs, 1H), 7.87 ppm (brd, 1H, $J = 9.1$ Hz); IR (KBr), 3300, 3100, 2980, 2950, 2890, 2600 (br), 1720, 1640, 1560, 1460, 1395, 1370, 1340, 1260, 1225, 1160, 1050, 930 cm^{-1}; mass spectrometry (*m/z*), calculated for $C_{14}H_{26}O_4N_2$ 286, found 287 $(M + H)^+$; $[\alpha]_D^{22} = -38.7°$ ($c = 1.00$, ethanol).

The chiral acid, glutaryl-L-valine *tert.*-butyl ester monocarboxylic acid, was prepared in a manner similar to that above. Coupling of the 4-benzyloxycarbonylbutanoyl N-4-hydroxysuccinimide ester and L-valine *tert.*-butyl ester hydrochloride [m.p. 144–146°C; $[\alpha]_D^{20} = +23.2°$ ($c = 0.41$, ethanol); lit[6]. m.p. 147–149°C], followed by hydrogenation gave the desired material: ^{1}H NMR (C^2HCl_3), δ 0.91 (d, 3H, $J = 6.8$ Hz), 0.94 (d, 3H, $J = 6.8$ Hz), 1.48 (s, 9H), 1.77–2.24 (m, 3H), 2.24–2.64 (m, 4H), 4.42–4.57 (dd, 1H), 6.48 (brd, 1H, $J = 9.4$ Hz), 7.62 ppm (brs, 1H); IR ($CHCl_3$; 0.07 *M*), 3480, 3400, 3280, 3140 (br), 2940, 2900, 2840, 1710, 1660, 1500, 1445, 1385, 1362, 1330, 1305, 1195, 1140, 1030, 835, 700 cm^{-1}; mass spectrometry (*m/z*), calculated for $C_{14}H_{28}O_5N$ 287, found, 288 $(M + H)^+$; $[\alpha]_D^{21} = -24.6°$ ($c = 1.00$, ethanol).

These chiral acids were ionically bonded in a 50 × 0.1 cm I.D. aminopropylsilica column by an *in situ* modification procedure described by Pirkle and Finn[7].

Preparation of CHBA and solutes

The chiral additive N-acetyl-L-valine *tert.*-butyl ester was prepared by treating the L-valine *tert.*-butyl ester hydrochloride with a mixture of acetic anhydride and triethylamine. Recrystallization from ethyl acetate gave a pure material of m.p. 106–106.5°C. Analysis: calculated for $C_{11}H_{21}O_3N$, C 61.37, H 9.83, N 6.51; found, C 61.45, H 9.99, N 6.49%; $[\alpha]_D^{18} = -29.9°$ (c = 1.03, ethanol). The preparation and characterization of the chiral additive N-acetyl-L-valine-*tert.*-butylamide have already been reported[2].

The N-acetylamino acid *tert.*-butyl esters used as solutes are the same as those described previously[2] except for the tyrosine derivative. The L-enantiomer and its racemate were prepared from the corresponding O-benzyltyrosine (Kokusan Chemical, Tokyo, Japan) according to the procedure described previously[2]. The tyrosine derivative was characterized as follows: N-acetyl-O-benzyl-DL-tyrosine *tert.*-butyl ester: m.p. 98–100°C [L-enantiomer, m.p. 93–96°C; $[\alpha]_D^{18} = +16.5°$ (c = 1.02, ethanol)]; ^{1}H NMR (C^2HCl_3), δ 1.40 (s, 9H), 1.96 (s, 3H), 3.02 (d, 2H, J = 5.9 Hz), 4.55–4.83 (m, 1H), 5.04 (s, 2H), 5.89 (brd, 1H), 6.83–7.13 (m, 4H), 7.39 ppm (s, 5H); IR (KBr), 3380, 3000, 2950, 1720, 1675, 1615, 1590, 1535, 1520, 1470, 1460, 1440, 1390, 1370, 1325, 1285, 1250, 1180, 1135, 1125, 1000, 970, 860, 815, 760, 715, 700 cm^{-1}; mass spectrometry (m/z), calculated for $C_{22}H_{27}O_4N$ 369, found 369 $(M)^+$.

RESULTS AND DISCUSSION

Outline of optical resolutions with a CHBA

Consider the solute enantiomers S_l and S_d, present at high dilution, in a silica gel column containing a CHBA. Chiral recognition depends on two categories of equilibrium relationships: a mobile and a stationary phase, in which the CHBA associates with each enantiomer. Assuming that diastereomeric hydrogen-bond associations consist in the formation of binary associated complexes, as has been shown in many instances, the following equilibrium expressions are given:

$$S_l + CHBA \overset{K_l}{\rightleftharpoons} C^*S_l$$

$$S_d + CHBA \overset{K_d}{\rightleftharpoons} C^*S_d$$

where C^*S_l and C^*S_d are complexes. These expressions allow us to recognize easily two possible origins of the observed resolution of enantiomers: a difference in stability between diastereomeric associations (*i.e.*, relative magnitudes of the equilibrium constants K_l and K_d) and a difference in chromatographic mobility of intact associated complexes overriding any stability difference.

If complex formation occurs predominantly in the mobile phase, the observed resolution may be ascribed to different association constants. That is, the more strongly complexed solute enantiomer is less strongly adsorbed on a stationary phase and is the first to be eluted. Complex formation by which enantioselectivity is attained may also occur from interaction between the solute and CHBA adsorbed on the silica gel surface. This selectivity, if any, may differ from that obtained in the mobile phase

bulk because of the surface responsible for adsorption of the molecules. Should the association stereoselectivity not be affected by the surface, then the more strongly complexed solute enantiomer is the last to be eluted.

The above mobile and stationary phase processes are superimposed on a scheme of competition between the CHBA in the mobile phase bulk and that adsorbed on the silica gel surface for the solute enantiomer. Assuming the competitive hydrogen-bond association to be present in the presently discussed chromatography, secondary partitioning of intact diastereomeric complexed solutes must be subordinated to the two-phase processes, as the complexed solute–CHBA interaction on the stationary surface is considered eventually to be resolved into the competitive association between the two CHBA for the solute. Therefore, in this paper, we discuss the chiral recognition mechanisms on the basis of partitioning of each solute enantiomer between the mobile and stationary phases.

Recognition mechanism in resolution with CHBA 1

CHBA 1 showed markedly stronger retention than the solute enantiomers in a silica gel column, as was evident from a comparison of the behaviour of CHBA 1

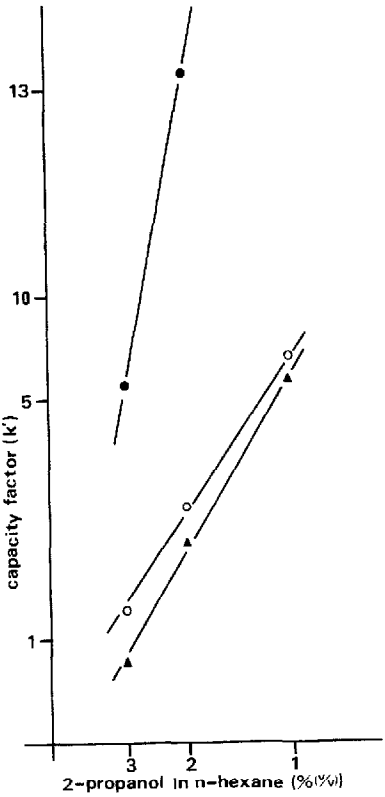

Fig. 1. Plot of the logarithm of the capacity factor (k') *versus* logarithm of the concentration of 2-propanol in *n*-hexane. ●, Chiral hydrogen-bonding additive (CHBA), N-acetyl-L-valine-*tert.*-butylamide (1); ○, CHBA, N-acetyl-L-valine *tert.*-butyl ester (2); ▲, N-acetyl-L-phenylalanine *tert.*-butyl ester, as a typical solute to be resolved. The k' value of CHBA 1 could not be determined when 2-propanol–*n*-hexane (1:99, v/v) was used, as the solute was not eluted within a reasonable time.

and the phenylalanine derivative, a typical solute. This comparison is shown in Fig. 1, which is a plot of the logarithm of the capacity ratios (k') *versus* the logarithm of the concentration of 2-propanol in *n*-hexane. This strong adsorptivity on the silica gel surface stems from the presence of the two amide units constituting the so-called C_5 and C_7 conformational sites (see Fig. 3).

CHBA 1 is, in fact, strongly adsorbed on the surface of the silica gel. The adsorbed concentration, calculated from measurement of the amount of additive in the column, was approximately 0.46 mmol/g of the support. The ratio of the additive content of the mobile phase bulk to that of the adsorbed layer in the column was 1:20 at 20°C when chloroform–*n*-hexane (40:60, v/v), containing 15 m*M* of CHBA 1, was employed as the mobile phase solvent. Table I gives the optical resolutions obtained under the above conditions for racemic N-acetylamino acid *tert.*-butyl esters containing aromatic moieties; this is a UV detection requirement. The observed elution order of the solute enantiomers was not affected by the chromatographic conditions, such as column temperature and mobile phase composition[2]: the D-enantiomers were eluted faster than the L-enantiomers in all the solutes examined. Fig. 2 shows a typical resolution of the racemic N-acetyl-O-benzyltyrosine *tert.*-butyl ester.

We attach much importance to the association that occurs on the adsorbed layer of CHBA 1. Our guiding principle is that the additive molecule should be firmly adsorbed by hydrogen bonding on the silica gel surface and that the solute enantiomers associate with the adsorbed layer without displacement of CHBA 1[8,9]. The CHBA should assume a position with its less hindered face towards the surface of the gel, leaving the face having the isopropyl side-chain connected to an asymmetric carbon open for the bulk of the mobile phase.

It seems reasonable to expect that this CHBA interacts with the solute enantiomers to form diastereomeric dimers whose associative interactions proceed by bidentate NH ··· O=C hydrogen bonds on a silica gel surface. CHBA 1 is capable of generating two structures of the hydrogen-bond associates: "C_5–C_7" dimers, in which

TABLE I

OPTICAL RESOLUTION OF THE ENANTIOMERS OF RACEMIC N-ACETYLAMINO ACID *TERT.*-BUTYL ESTERS WITH CHIRAL HYDROGEN BONDING ADDITIVE (CHBA), N-ACETYL-L-VALINE-*TERT.*-BUTYLAMIDE (1) ON A SILICA GEL COLUMN

Chromatographic conditions: column, 25 × 0.4 cm I.D.; packing, LiChrosorb Si 60 (5 μm); mobile phase solvent, chloroform–*n*-hexane (40:60, v/v), containing 15 m*M* of CHBA 1, N-acetyl-L-valine-*tert.*-butylamide; flow-rate, 1 ml/min; column temperature, 20°C; detection, UV at 265 nm.

Amino acid	*Capacity factor (k')*		*Separation factor (α)**
	D-	*L-*	
N-*t*-BuTrp	3.31	4.07	1.23
S-BzlCys	3.57	4.28	1.20
α-PheGly	4.22	5.10	1.21
Phe	4.30	5.56	1.29
O-BzlTyr	4.31	5.13	1.19

* $\alpha = k'_L/k'_D$.

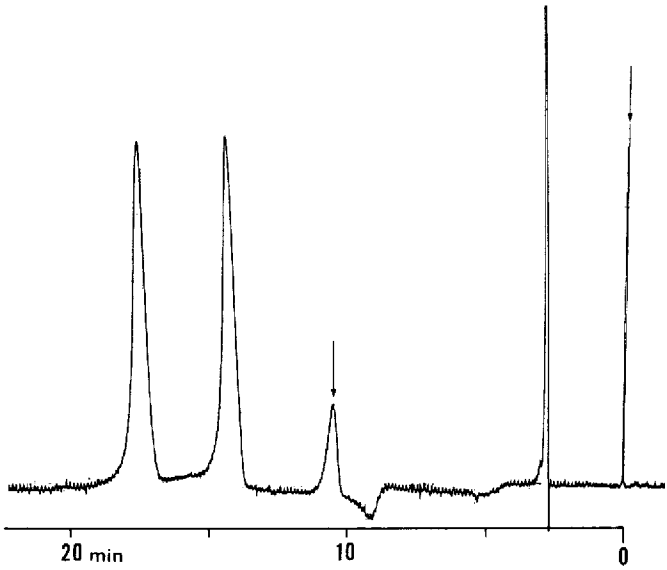

Fig. 2. Optical resolution of racemic N-acetyl-O-benzyltyrosine *tert.*-butyl ester with CHBA 1, N-acetyl-L-valine-*tert.*-butylamide. Chromatographic conditions: mobile phase solvent, chloroform–*n*-hexane (40:60, v/v), containing 15 m*M* of CHBA 1; flow-rate, 1 ml/min; column temperature, 20°C; detection, UV 250 nm; sample volume injected, 3 μl of 2% (v/v) chloroform solution. The order of emergence of the enantiomers was such that the D-enantiomer was eluted faster than the L-enantiomer. The peak indicated with an arrow was assumed to correspond to CHBA 1, displaced by the solute, because of its appearance at a k' value of 2.81, regardless of the solutes to be resolved. This displaced additive cannot be a primary layer, firmly attached the surface of the silica, but the additive hydrogen-bonded to the primary layer.

the C_7 conformational site is offered by CHBA 1, and "C_5–C_5" dimers. These two dimers can be formed as the solute enantiomers pass through the column. The associated complex of the enantiomers may either lie flat on the surface or stand upright because the association competes in part with the adsorption of the CHBA. These two orientations may possibly be in equilibrium, rather than one of the two predominating. Assuming the above associations to occur in the adsorbed state of CHBA 1, as shown in Fig. 3, the associated complex of the L-enantiomers can adopt a more stable flat-lying posture, because their side-chains are oriented towards the bulk of the mobile phase, similar to that of CHBA 1. The D-enantiomers must, however, orient their large substituents towards the surface of the silica gel when in the fully hydrogen-bonded state and their probable lesser degree of adsorption may result in elution of the D-enantiomers first.

The above selectivity is presumed to be exerted by the silica gel surface on which CHBA is deposited, and hence may be independent of the "raw" enantioselectivity, *i.e.*, the difference in stability between the diastereomeric associated complexes observed in solution. This selectivity therefore must be termed "chromatographic enantioselectivity", as a direct allocation of raw enantioselectivity cannot be made for the stationary phase process, although it must be kept in mind that the selectivity contributes, at least in part, to the overall stationary phase process.

Immobilization of CHBA on the silica gel surface should make it possible to

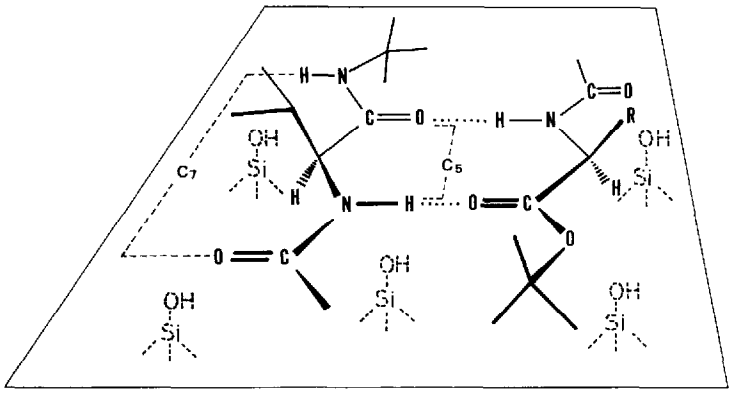

Fig. 3. The solute L-enantiomer–additive association occurring in the adsorbed state of CHBA 1, N-acetyl-L-valine-*tert.*-butylamide on a silica gel surface. The complexed L-enantiomers, whose associative interactions are bidentate NH · · · O=C hydrogen bonds, can provide a more stable flat-lying orientation because their side-chains are oriented toward the bulk of the mobile phase, similar to that of CHBA 1. The "C_5–C_7" dimers in which the C_7 conformational site is offered by CHBA 1 may be formed along with the "C_5–C_5" dimers in this figure, as the solute enantiomers pass through the column.

elicit the enantioselectivity of the hydrogen-bonded phase from the chiral mobile phase system. We therefore prepared the chiral stationary phase CSP 1, shown in Fig. 4, consisting of glutaryl-L-valine-*tert.*-butylamide monocarboxylic acid, containing the CHBA 1 structure ionically bound to 3-aminopropylsilica, and made an examination to determine whether the solute enantiomers are resolved in the same elution order as that by CHBA 1. CSP 1 showed, as expected, the same elution order, *i.e.*, the D-enantiomer is followed by the L-enantiomer, as shown in Table II. It seems reasonable to expect a recognition mechanism similar to that assumed for CHBA 1. The chiral moiety of the L-configuration is also adsorbed on the surface and forms the most stable associated complex with the L-enantiomer of the solute. The mobile phase solvent used contained not chloroform but 2-propanol as the stronger solvent component, as chloroform lacks the capability to elute the amino acid solutes in the absence of CHBA 1. It is evident that the elution order is independent of the mobile phase composition in chiral stationary phases, on the basis of hydrogen-bond associations, as has previously been reported[10].

The actual resolutions, expressed as the separation factor (α), are determined by superimposing the raw enantioselectivity in the mobile phase process on the above stationary phase process. Chiral recognition in the mobile phase is perhaps not great enough to surpass the stereoselectivity of the stationary phase, as is implied by a comparison of the elution order and magnitude of the resolutions observed for CSP

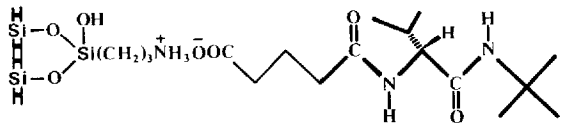

Fig. 4. Aminopropylsilica, modified *in situ* by glutaryl-L-valine-*tert.*-butylamide monocarboxylic acid (CSP 1).

TABLE II

OPTICAL RESOLUTION OF THE ENANTIOMERS OF RACEMIC N-ACETYLAMINO ACID *TERT.*-BUTYL ESTERS ON THE PACKED AMINO COLUMN, MODIFIED BY GLUTARYL-L-VALINE-*TERT.*-BUTYLAMIDE MONOCARBOXYLIC ACID

Chromatographic conditions: column, 50 × 0.1 cm I.D.; packing, 3-aminopropyl-silica [Nucleosil-5NH_2 (5 μm)], *in situ* modified by the chiral acid glutaryl-L-valine-*tert.*-butylamide monocarboxylic acid (CSP 1); mobile phase solvent; 2-propanol–*n*-hexane (1:99, v/v); flow-rate, 60 μl/min; column temperature, 20°C; detection, UV at 254 nm.

Amino acid	*Capacity factor (k')*		*Separation factor (α)**
	D-	*L-*	
N-*t*-BuTrp	5.79	6.88	1.19
S-BzlCys	4.98	5.59	1.12
α-PheGly	6.10	6.81	1.12
Phe	5.50	6.43	1.17
O-BzlTyr	7.17	8.26	1.15

* $\alpha = k'_L/k'_D$.

1 and those for CHBA 1, although clarification as to whether the mobile phase process enhances or reduces the selectivity in the stationary surface was not possible in the present study.

Recognition mechanism of resolution with CHBA 2

CSP 2, obtained by substitution of the C-terminal amide unit with an ester unit, as shown in Fig. 5, was insensitive to solute enantiomers at the column temperatures used for CSP 1, such as 20°C. A lower column temperature of −27°C forced the resolution of the racemic solutes on the CSP 2 column in the same elution order as that observed for CSP 1. For example, racemic N-acetylvaline *tert.*-butyl ester was resolved at an α value of 1.11 (k'_D = 2.37, k'_L = 2.63), using dichloromethane–*n*-hexane (60:40, v/v) as the mobile phase solvent. Detection was effected by UV absorption at 230 nm. When CHBA 2, which is believed to be the immobilized moiety in CSP 2, was used instead of CHBA 1, the resolutions appeared to be induced by raw enantioselectivity in the mobile phase process, as the selectivity exerted by this molecule adsorbed on the silica gel surface need not be taken into consideration at ambient temperatures.

CHBA 2 is capable of resolving the solute enantiomers at a concentration of 0.1 *M* in chloroform–*n*-hexane (10:90, v/v), as shown in Table III. The observed order of emergence of the solute enantiomers was reverse of that obtained with

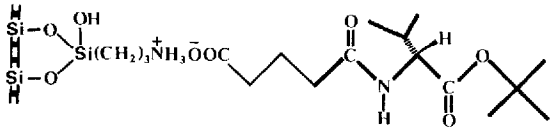

Fig. 5. Aminopropylsilica, modified *in situ* by glutaryl-L-valine *tert.*-butyl ester monocarboxylic acid (CSP 2).

TABLE III

OPTICAL RESOLUTION OF THE ENANTIOMERS OF RACEMIC N-ACETYLAMINO ACID *TERT.*-BUTYL ESTERS WITH CHBA, N-ACETYL-L-VALINE-*TERT.*-BUTYL ESTER (2) ON A SILICA GEL COLUMN

Chromatographic conditions: column, 25 × 0.1 cm I.D.; packing, Spherosil XOA-600 (5 μm); mobile phase solvent, chloroform–*n*-hexane (10:90, v/v), containing 0.1 *M* of CHBA 2; flow-rate, 30 μl/min; column temperature, 20°C; detection, UV at 254 nm.

Amino acid	*Capacity factor (k')*		*Separation factor (α)**
	L-	*D-*	
N-*t*-BuTrp	9.51	10.73	1.13
S-BzlCys	9.03	10.08	1.12
α-PheGly	9.31	10.43	1.12
Phe	9.85	11.46	1.16
O-BzlTyr	12.49	15.24	1.22

* $\alpha = k'_L/k'_D$.

CHBA 1: the L-enantiomers were eluted faster than the D-enantiomers. NMR and IR spectra of the self-association of CHBA 2 in solution can thus be studied as an analogue of the solute–additive associations in the mobile phase in order to clarify the chiral recognition mechanism responsible for the enantioselectivities of CHBA 2. Diastereomeric associated complexes, similar to those observed in CHBA 2, are probably formed from pairs of alternate solutes and CHBA 2.

A concentration study of ^1H and ^{13}C NMR in carbon tetrachloride solutions showed that the diastereomeric dimers, as depicted in Fig. 6, whose associative interactions are bidentate NH ··· O=C (ester) hydrogen bonds, are formed in enantiomeric solutions[11]. The formation of these dimers in enantiomerically enriched mixtures is also indicated by a split of the amide NH resonance into two ^1H NMR signals for the D- and L-enantiomers, termed self-induced non-equivalence[12,13], in 0.1 *M* carbon tetrachloride solution.

The IR spectra confirm the stability difference between the homochiral (Fig. 6a) and the heterochiral hydrogen-bonded dimer (Fig. 6b) by an external comparison of the optically pure and racemic samples in carbon tetrachloride solution. In the

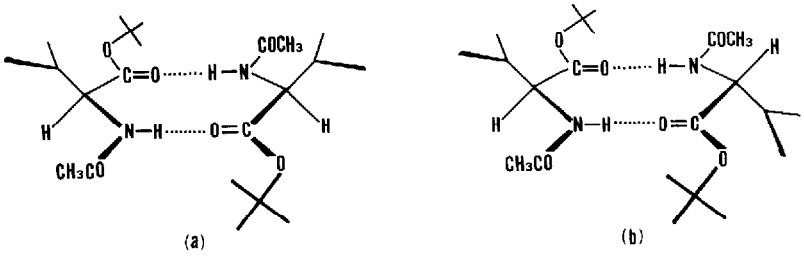

Fig. 6. Diastereomeric dimers interlinked via bidentate NH ··· O=C (ester) hydrogen bonds, proposed to account for the self-induced NMR non-equivalence of the enantiomeric carbon tetrachloride solution of the chiral N-acetylvaline *tert.*-butyl ester (2). The homochiral (L–L) dimer (a) is more stable than the heterochiral dimer (b).

L-enantiomeric solution, the intensity of the hydrogen-bonded NH stretching band (3360 cm^{-1}), corresponding to the intermolecular hydrogen bond[14] in the amide group, is stronger than that in the racemic mixture containing enantiomeric sample at concentrations exceeding 0.01 *M* at *ca.* 23°C. The homochiral hydrogen-bonded dimer formed in the enantiomerically pure solution was concluded to be more stable than the corresponding heterochiral dimer in the racemic solution. This stability difference is not affected by replacement of the co-solvent carbon tetrachloride with chloroform–hexane (10:90, v/v), as is indicated by the external comparison of intensity on the NH absorption, and hence the elution order observed with CHBA 2 is clearly explained in terms of the dominance of the mobile phase process: the most strongly complexed solute enantiomer, *i.e.*, the L-enantiomer in this instance, in the mobile phase is less strongly adsorbed on the stationary phase and is the first to be eluted.

The contrast in elution order between CHBA 1 and CHBA 2 is at least in part ascribable to the structure of CHBA 2, having only one C_5 conformational site for bidentate binding to the solute enantiomers. That is, surrender of the C_5 site to the solutes is strongly competitive with the adsorption of CHBA 2 itself.

It is of interest that all chiral hydrogen-bonding agents capable of inducing the formation of distereomeric solvates with enantiomeric molecules containing proton--releasing or proton-accepting groups are potentially capable of serving as chiral mobile phase additives, provided that there are appropriate means for detecting the elution of the solute enantiomers.

CONCLUSIONS

Chiral mobile phase additives provide a chiral environment in the column and form an associated complex with each enantiomer. Hence there are two different chiral recognition mechanisms responsible for the enantioselectivity of the CHBA. If the CHBA is firmly adsorbed on the silica gel surface and acts as a chiral hydrogen-bonded phase with which the solutes can form diastereomeric associated complexes such as CHBA 1, the main driving force for the chiral recognition can be the steric effect exerted by the surface of the support adsorbing the solute–additive associates rather than the difference in stability between the complexes in the mobile phase bulk. This enantioselectivity must be termed "chromatographic enantioselectivity". However, if the CHBA adsorbed on the surface is displaced by the solutes or its adsorbed state cannot form diastereomeric associated complexes capable of generating optical resolutions of the solutes, such as CHBA 2, raw enantioselectivity in the mobile phase becomes the most important source in the chiral resolution. In this instance, the chromatography can function as a sophisticated means for clarification of the association stereoselectivity of chiral molecules.

ACKNOWLEDGEMENTS

We gratefully acknowledge the capable assistance of Naoyuki Saito and Yuuichi Motoyama in carrying out this research.

REFERENCES

1 A. Dobashi and S. Hara, *Tetrahedron Lett.*, 24 (1983) 1509.
2 A. Dobashi and S. Hara, *J. Chromatogr.*, 267 (1983) 11.
3 A. Dobashi and S. Hara, *Anal. Chem.*, 55 (1983) 1805.
4 Y. Dobashi, A. Dobashi and S. Hara, *Tetrahedron Lett.*, 25 (1984) 329.
5 Y. Dobashi and S. Hara, *J. Am. Chem. Soc.*, 107 (1985) 3406.
6 G. W. Anderson, J. E. Zimmerman and F. M. Callahan, *J. Am. Chem. Soc.*, 46 (1964) 2935.
7 W. H. Pirkle and J. M. Finn, *J. Org. Chem.*, 46 (1981) 2935.
8 R. P. W. Scott and P. J. Kucera, *J. Chromatogr.*, 149 (1978) 93.
9 R. P. W. Scott, *Faraday Symp. Chem. Soc.*, 15 (1981) 49.
10 A. Dobashi, K. Oka and S. Hara, *J. Am. Chem. Soc.*, 102 (1980) 7122.
11 A. Dobashi, N. Saito, Y. Motoyama and S. Hara, *J. Am. Chem. Soc.*, in press.
12 W. H. Pirkle and D. J. Hoover, in E. L. Eliel and N. L. Allinger (Editors), *Topics in Stereochemistry*, Vol. 13, Wiley-Interscience, New York, 1982, p. 316.
13 G. R. Weisman, in J. D. Morrison (Editor), *Asymmetric Synthesis*, Vol. 1, Academic Press, New York, 1983, p. 153.
14 R. T. Ingwall, C. Gilon and M. Goodman, *Macromolecules*, 9 (1976) 802.