ELSEVIER

International Journal of Mass Spectrometry 193 (1999) 123–130

International Journal of Mass Spectrometry

Determination of enantiomeric excess for organic primary amine compounds by chiral recognition fast-atom bombardment mass spectrometry

Masami Sawada[a,*], Hiroshi Yamaoka[b], Yoshio Takai[a], Yorito Kawai[c], Hitoshi Yamada[a], Tomoko Azuma[b], Tamaki Fujioka[b], Toshikazu Tanaka[c]

[a]*Materials Analysis Center, the Institute of Scientific and Industrial Research, Osaka University, Mihogaoka, Ibaraki, Osaka 567-0047, Japan*
[b]*Department of Natural Science, Osaka Women's University, Daisen-cho, Sakai, Osaka 590-0035, Japan*
[c]*Faculty of Engineering, Osaka Institute of Technology, Asahi-ku, Osaka 535-0002, Japan*

Received 15 May 1999; accepted 16 July 1999

Abstract

Enantiomeric excess (ee) of organic primary amine compounds such as phenylglycine methyl ester hydrochloride (**2**) has been determined by fast-atom bombardment (FAB) mass spectrometry (NBA matrix). Chiral recognition in host–guest complexation systems between crown ethers [H] and amino acid ester ammonium ions [G] has been extended to the ee determination. The method characteristically uses a 1/1 mixture of a pair of enantiomeric hosts whose enantiomer is isotopically labeled [(RRRR)-**1** and (SSSS)-**1**-d_6]. Chiral recognition of a given guest is simply measured with the given host–pair reagent from the relative peak intensities of the two corresponding diastereomeric host–guest complex ions in $I[(H_{RRRR} \cdot G)^+]/I[(H_{SSSS\text{-}d_6} \cdot G)^+ = I_R/I_{S\text{-}d_6}$, so called IRIS value. The IRIS value varies in a linear fashion with the ee quantitiy of **2** and produces a symmetric linear V-shaped plot, indicating that in the case of a primary amine guest (such as **2**) with unknown ee, one can determine the ee by this type of chiral recognition FAB mass spectometry. Further, based on the observed concentration effects on the IRIS values, it is suggested that the present IRIS value reflects the concentration ratio of the diastereomeric complex ions formed in the matrix. (Int J Mass Spectrom 193 (1999) 123–130) © 1999 Elsevier Science B.V.

Keywords: Chiral recognition; FAB mass spectrometry; Enantiomeric excess; Isotope labeling; Host-guest complexation

1. Introduction

It is important for not only the structural elucidation of clinical, pharmaceutical, and agricultural chemicals, but also other asymmetric organic synthesis processes to determine the enatiomeric excess (ee) or optical purity of the given samples. Instead of the old fashioned polarimetry where a large amount of sample is generally needed, nowadays some typical methods are used, for example, chiral separation by high performance liquid chromatography. The injection of an enantiomeric mixture onto the chiral stationary phased column gives two isolated peaks which have different retention times from each other on the

* Corresponding author. E-mail: m-sawada@sanken.osaka-u.ac.jp

PII S1387-3806(99)00166-9

same chromatogram. Similarly, capillary electrophoresis using chiral additives is covered by the same category. On the other hand, in the case of nuclear magnetic resonance (NMR) spectrometry, treatment of a given sample consisting of enantiomers with a chiral derivatizing reagent (or a chiral solvation reagent) gives slightly different chemical shifts in the NMR spectra [1–4].

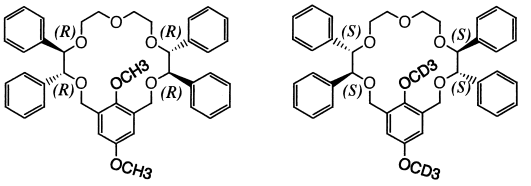
Mass spectrometry is a highly sensitive method. Yet, it has been scarcely noted as the ee determination tool for chiral organic amines because of the absence of mass difference between diastereomeric isomers on the mass spectra. To our knowledge, there have been only two reports where the possibilities of the ee determination (1) in the ethyl tartrate system and (2) in the binaphythyl system were suggested by the detections of (1) a proton bound dimer ion [5] and (2) a proton bound association ion (1-phenylethyl amine-binaphthyl derivative) [6] using fast atom bombardment (FAB) ionization.

However, recently the chiral recognition ability of designed hosts toward amino acid guests could be effectively detected by FAB mass spectrometry based upon host–guest chemistry [7–12]. We call this approach chiral recognition mass spectrometry. Herein, isotope labeling for one of the enantiomeric guests had been used to effectively distinguish between diastereomeric host–guest complex ions on the same mass spectra (the enantiomer labeled guest method or the EL–guest method). Thus, the EL-guest method provides a simple and quick determination for the chiral recognition ability of crown ether hosts toward chiral amino acid guests, compared with the NMR titration method where it takes long time to execute.

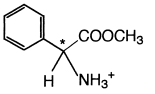
Here, we describe a novel ee determination method for organic primary amine compounds as the extended application of chiral recognition FAB mass spectrometry. For the present purpose, isotope labeling is necessary for one of the two enantiomers in the chiral crown ether hosts (not in the chiral amine guests). We call this the enantiomer labeled host method (the EL–host method). This is the first example for the ee determination of amine compounds by the FABMS/EL–host method [13].

2. Experimental

2.1. Materials



(*RRRR*)-**1** (*SSSS*)-**1**-d_6



2

Scheme 1.

Chiral hosts, (RRRR)-**1** and (SSSS)-**1**-d_6, were prepared by previously reported procedures [7–13]. In the case of the deuterium-labeled compounds, 2,6-bis(bromomethyl)hydroquinone d_6-dimethyl ether was obtained from 2,6-dimethylhydroquinone by CD_3I and subsequent side chain bromination [13]. Chiral guests, (R)-**2** and (S)-**2**, were prepared by the esterification of the corresponding chiral amino acids purchased from Aldrich (Milwaukee, WI) and Sigma (Milwaukee, WI), respectively [7]. *m*-Nitrobenzyl alcohol (NBA) was purchased from Aldrich.

2.2. FAB mass spectrometry

All the FAB mass spectra were acquired with a JMS-DX300 (JEOL, Akishima, Tokyo, Japan), EB double-focusing instrument equipped with a standard JEOL FAB ionization source fitted with a xenon gun. The conditions are following: acceleration of xenon beam, 6 kV; mode, positive; matrix, NBA; ion source pressure, typically 10^{-5}–10^{-6} Torr; acceleration voltage, 3 kV; emission current, 20 mA; scan rate, 5 s/scan (mass range to *m/z* 1000). Data acquisition was done using a JEOL JMA 5000 data processing system.

2.3. Preparation of typical sample solutions for the FABMS/EL–host method

Generally, a weighed sample in a microtube was dissolved with an appropriate amount of solvent using a microsyringe. Sampling procedures were as follows. (1) 32.2 μL of chloroform was added to 4.26 mg of host (RRRR)-**1** (solution H_R) (0.16 M), (2) 31.1 μL of chloroform was added to 3.15 mg of host (SSSS)-**1**-d_6 (solution H_S) (0.16 M), (3) 25 μL of the solution H_R was added to 25 μL of the solution H_S (solution 1) (0.08 M in each enantiomer), (4) 40.3 μL of methanol was added to 0.87 mg of guest **2** (solution 2) (0.080 M).

A final sample solution for the FABMS/EL–host method was typically mixed with (1) 5 μL of the solution 1, (2) 5 μL of the solution 2, and (3) 30 μL of NBA using a microsyringe and an ultrasonic processor (for 1–5 h). After evaporation of chloroform and methanol, the concentrations in NBA were as follows: $[H_{RRRR}] = [H_{SSSS}]$-$d_6 = [G] = 0.0133$ M ([H]:[G] = 2:1). The mixed solution stood overnight and the 1 μL aliquot was put on a FAB probe to measure the mass spectra.

2.4. Determination of ee values

Four relative intensity data obtained from the 10th, 20th, 30th, and 40th scan data were averaged ($n = 4$) and tabulated in Tables 1–3. No isotope correction for natural abundance was done because of a 6 mass - unit difference between the diastereomeric complex ions.

3. Results

3.1. Concept of the EL–host method

We chose the chiral crown ether **1** as the host compound and synthesized the enantiomeric pair of unlabeled (RRRR)-**1** and the enantiomer-labeled (SSSS)-**1**-d_6. In the latter compound, methoxy groups on the benzene ring are deuterium labeled as OCD_3. Thus, an equimolar mixture of (RRRR)-**1** and (SSSS)-**1**-d_6 was used as the host–pair reagent for the ee determination of organic amine samples.

As the guest, we chose phenylglycine methyl ester hydrochloride (**2**) because of its relatively strong complexation ability with host **1** [7]. Several guest solutions of **2** with different ees were prepared by the appropriate mixing of (R)-**2** and (S)-**2** (both have 100% ee).

The host–pair reagent (H_{RRRR}-**1** / H_{SSSS}-**1**-d_6 = 1/1) was mixed with guest **2** to do the FABMS measurements. Two diastereomeric host–guest complex ions simultaneously appeared in one FAB mass spectrum. The relative peak intensity (IRIS value)

Table 1
Concentration effects on IRIS values for the FABMS/EL–host method[a] {[H] = 0.0167 M = constant and [G] = varied}

[H]:[G]	IRIS value	$[H_{RRRR} \cdot G]/[H_{SSSS} \cdot G]^{Calc\ b}$
1:6[c]	1.32	1.35
1:3	1.45	1.53
1:1	1.76	1.78
2:1	1.85	1.88
6:1[d]	1.86	1.95

[a] The combination of host [(RRRR)-**1**: (SSSS)-**1**-d_6 = 1:1] with guest (R)-**2** (100% ee). [H] means a concentration of sum of the two enantiomeric hosts. Numerical concentration exhibited is the concentration in NBA after evaporation of MeOH and $CHCl_3$ solvents in an ion source.

[b] Calculated concentration ratio of the diastereomeric host–guest complex ions in NBA. See Sec. 4.1.

[c] [G] = 0.100 M.

[d] [G] = 0.0032 M.

Table 2
Concentration effects on IRIS values for the FABMS/EL–host method[a] {[H]/[G] is kept constant as 2.0}

$[G]_M$	IRIS value	$[H_{RRRR} \cdot G]/[H_{SSSS} \cdot G]^{Calc\ b}$
0.040	1.76	1.70
0.0267	1.79	1.75
0.0133	1.84	1.83
0.010	1.93	1.86
0.0067	1.98	1.90

[a] The combination of host [(RRRR)-**1**: (SSSS)-**1**-d_6 = 1:1] with guest (R)-**2** (100% ee). [H] means a concentration of sum of the two enantiomeric hosts.

[b] Calculated concentration ratio of the diastereomeric host–guest complex ions in NBA. See Sec. 4.1.

Table 3
Change in IRIS value based on change in ee value of guest **2**[a]

Prepared ee value of **2**[b] (%)	IRIS value[c]
(R)-100	1.81
(R)-80	1.60
(R)-50	1.33
(R)-20	1.08
0	0.98[d], 0.96
(S)-20	0.91
(S)-50	0.71
(S)-80	0.62
(S)-100	0.53

[a] [H] = 0.0267 M, [G] = 0.0133 M in NBA.
[b] Prepared by mixing appropriate amounts of (R)-**2** and (S)-**2**.
[c] An averaged value of 10th, 20th, 30th, and 40th scan data (n = 4).
[d] IRIS = 0.98 ± 0.04 (n = 30).

was expected to be a measure of the chiral recognition properties.

$$I[(H_{RRRR} \cdot G)^+]/I[(H_{SSSS}\text{-}d_6 \cdot G)^+]$$
$$= I_R/I_S\text{-}d_6 = \text{IRIS (abbreviation)}$$

where I means the peak intensity of the corresponding complex ions in the FAB mass spectra.

The fundamental concept of the EL–host method is schematically shown in Fig. 1, where the optically pure (R)–guest complexes the (RRRR)–host by an arbitrary factor of 2.0 as strongly as the (SSSS)–host (run 1, IRIS = 2.0). Therefore, the optically pure (S)–guest should complex the (SSSS)–host by a factor of 2.0 as strongly as the (RRRR)–host (run 2, IRIS = 0.50) because of the mirror image relationship during the host–guest complexation. Furthermore, the racemic (RS)–guest (0% ee) should provide a pair of equal peak intensities (run 3, IRIS = 1.0) due to the net compensation of a racemic host/racemic

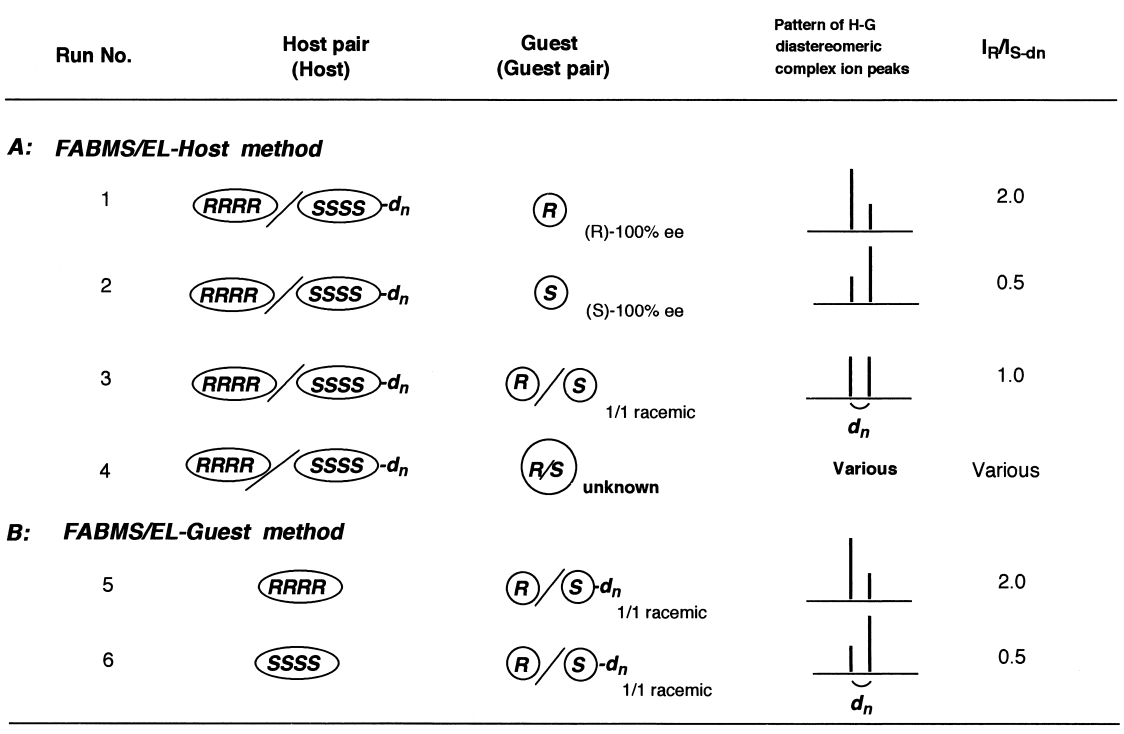


Fig. 1. Schematic fundamental concept of the enantiomer-labeled host and guest methods.

guest combination in such competitive systems. Accordingly, it is noteworthy that in the case of a given guest sample with unknown ee, one can expect to determine the ee (percent enantiomeric excess) from the relationship between the IRIS and the ee values (calibration line).

In this article, we characteristically focus on the relative peak intensities of the diastereomeric host–guest complex ions as a quantitative measure [14–16]. A pair of diastereomeric complex ions have the same molecular weights, formula, and functional groups. They have only a slight difference in the three dimensional structure. It can be reasonably assumed that the two ions have almost the same transferability from the matrix to the gas phase during the FAB ionization process. Therefore, it is noted that the present system should be an ideal case where the relative peak intensity reflects the relative concentration of the pre-formed diastereomeric ions in the matrix.

3.2. Concentration conditions (concentration effect on IRIS values)

Table 1 and Fig. 2(a) show the change in IRIS values due to the change in concentration of the optically pure guest (R)-**2** in which the concentration of the host-pair reagent **1** is constant. The observed IRIS values appreciably increased from 1.3 to 1.9 with the decreasing change in the concentration of [G] (also with the increasing change in the [H]/[G] value), although the intensities of the diastereomeric complex ions themselves have decreased. Judging from a balance between the quality of the mass spectrum (peak intensity and scan stability) and the degree of the detected chiral recognition ability, we set up the concentration ratio as [H]:[G] = 2:1.

On the other hand, Table 2 and Fig. 2(b) show the change in IRIS values due to the simultaneous change in the concentrations of [H] and [G], maintaining a constant ratio as [H]:[G] = 2:1. The observed IRIS values also appreciably increased from 1.7 to 1.9 with the decreasing change in the concentrations of [H] and [G]. The lower the concentrations, the higher the degree of the mass spectrometrically detected chiral recognition (thermodynamically detected chiral recognition, K_R/K_S = 2.0, see Sec. 4.1). Judging from a balance between the quality of the mass spectra and the degree of the detected chiral recognition ability, we set up the optimum concentration conditions as [H] = 0.0267 M and [G] = 0.0133 M ([H]/[G] = 2.0).

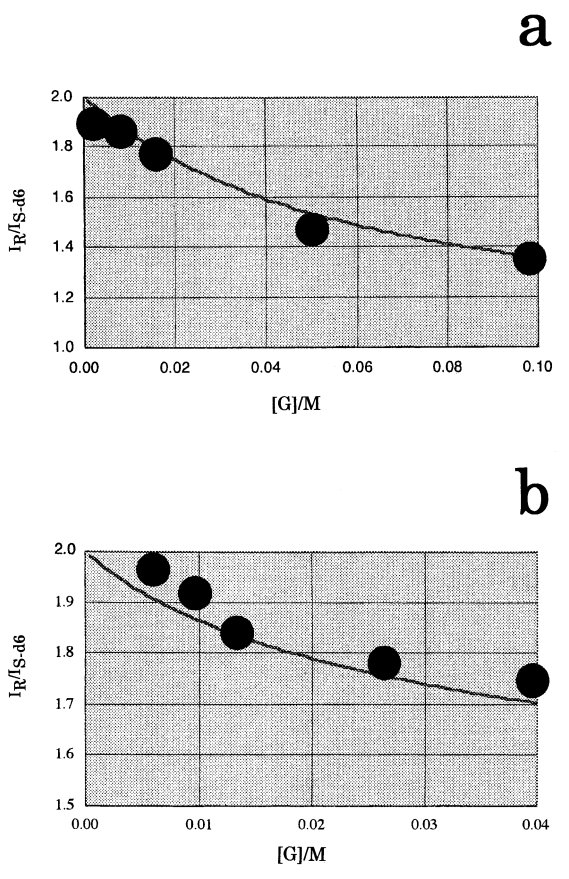


Fig. 2. (a) A plot of IRIS values vs. concentration of (R)-**2** (100% ee) in the enantiomer-labeled host method (constant concentration of the host–pair reagent **1**). (b) A plot of IRIS values vs. concentration of (R)-**2** (100% ee) in the enantiomer-labeled host method ([H]/[G]= 2.0; fixed ratio).

3.3. Change in IRIS value based on change in ee value

Several guest solutions with different ees were prepared by the appropriate mixing of solutions of the optically pure (R)- and (S)-**2**. The IRIS values were determined by the FABMS/EL–host method using the host–pair reagent [(RRRR)-**1** and (SSSS)-**1**-d_6].

Typical FAB mass spectra are shown in Fig. 3, and the results are summarized in Table 3. The observed IRIS value approaches to unity (1.0) with decreasing ee value. In order to check a symmetrical feature, the IRIS values are plotted against the ee values when the (R)–guest is in excess, and the reciprocal of the IRIS values (1/IRIS) are plotted against the ee values when the (S)–guest is in excess (Fig. 4). Obviously, the IRIS value varies in a linear fashion with the ee quantity and produces a symmetric V-shaped plot.

4. Discussion

4.1. Correlation between IRIS values and concentration ratios of pre-formed diastereomeric complex ions under competitive equilibrium conditions

We can now simply consider the following competitive equilibrium system of equations:

$$H_1 + G \underset{}{\overset{K_1}{\rightleftharpoons}} (H_1 \cdot G) \quad (1)$$

$$H_2 + G \underset{}{\overset{K_2}{\rightleftharpoons}} (H_2 \cdot G) \quad (2)$$

Under competitive equilibrium conditions, the following third order equation referring to [G] can be derived [7, 8, 17].

$$K_1K_2[G]^3 - \{K_1K_2([G]_0 - [H_1]_0 - [H_2]_0) - K_1 - K_2\}[G]^2 - \{K_1([G]_0 - [H_1]_0) + K_2([G]_0 - [H_2]_0) - 1\}[G] - [G]_0 = 0 \quad (3)$$

Here, the letter [G] means the concentration of the free chiral guest, which does not complex with the chiral hosts. The letter $[G]_0$ means an initial concentration of G. If the three initial concentrations, $[H_1]_0$, $[H_2]_0$, $[G]_0$, and the two equilibrium constants, K_1 and K_2, are given, the concentrations of the diastereomeric complex ions, $[H_1 \cdot G]$ and $[H_2 \cdot G]$, can be calculated.

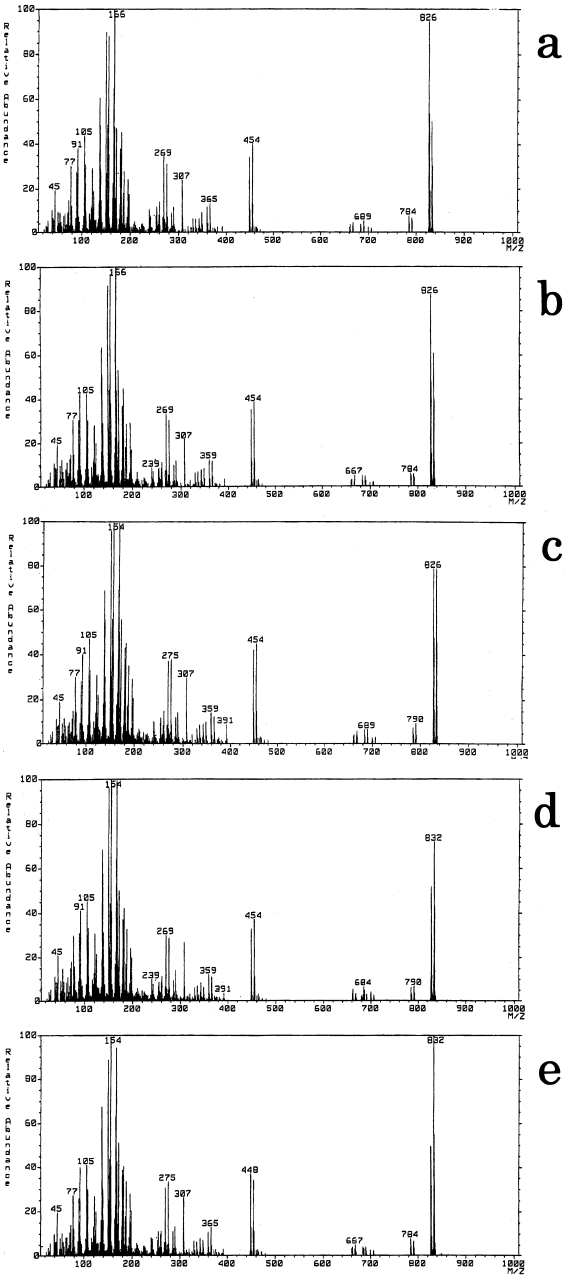


Fig. 3. Typical FAB mass spectra (NBA matrix) for the complexation between host-reagent **1** [a 1/1 mixture of (RRRR)-**1** and (SSSS)-**1**-d_6] and guest **2** with various ee quantities using the enantiomer-labeled host method: (a) (R)-100% ee, (b) (R)-50% ee, (c) 0% ee (racemic), (d) (S)-50% ee, and (e) (S)-100% ee.

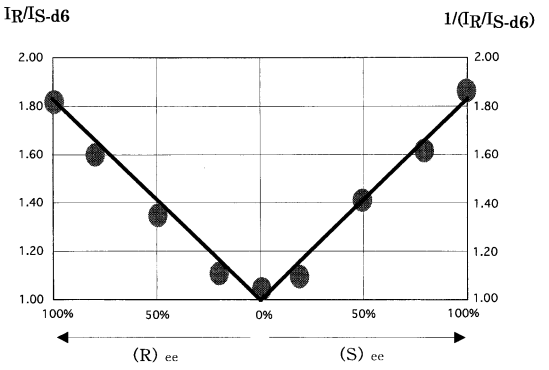


Fig. 4. A plot of IRIS values (or 1/IRIS values) against ee quantities of guest **2** (calibration line).

The following equilibrium constants had been determined in $CD_3OD/CDCl_3$ (10/1 by volume percent) at 25 °C using NMR titration methods [7]:

host (RRRR)-**1** with guest (R)-**2**, $K_R = 2.0\ M^{-1}$

host (RRRR)-**1** with guest (S)-**2**, $K_S = 1.0\ M^{-1}$

Based upon the stereochemical viewpoints (cross-chiral correlations in Fig. 1), the equilibrium constant of host (SSSS)-**1** with guest (R)-**2** should be assumed as $1.0\ M^{-1}$. Of course, large solvent effects have been generally observed in the magnitude of equilibrium constants (K_R and K_S). However, when the ratio value, (K_R/K_S), is treated as used for a measure of chiral recognition of host **1** toward guest **2**, the solvent effect becomes much smaller and may be mostly cancelled [18]. Although the corresponding K_R/K_S values in NBA are not available, we tentatively assumed as the same as the above: that is $K_R/K_S = 2.0$ in NBA (e.g. $K_1 = 20\ M^{-1}$ and $K_2 = 10\ M^{-1}$ in eq. (3) due to the lesser polar solvent employed). Then, we can calculate the $[(H_{RRRR} \cdot G)^+]/[(H_{SSSS} \cdot G)^+]$ value using the concentrations of the measuring sample for FABMS [Tables 1, 2 and Fig. 2(a)(b)].

Our experimental data (IRIS values) obtained by FABMS are listed in Tables 1 and 2. The IRIS values obtained by the EL–host method are very close to the calculated $[(H_{RRRR} \cdot G)^+]/[(H_{SSSS} \cdot G)^+]$ values. These findings have suggested that the concentration ratio of the pre-formed diastereomeric ions in the matrix are quantitatively or semiquantitatively detected by the corresponding relative peak intensity, at least as far as the diasteromeric host-guest complex ions are concerned. In other words, it has been demonstrated that chiral recognition ability in solution, which is needed for analytical/organic chemists, can be easily deduced from the IRIS value using the chiral recognition FAB mass spectrometry under our concentration conditions employed.

4.2. Determination of ee from IRIS values

As shown in Fig. 4, the ee value is correlated with the IRIS value as a symmetrical V-shaped plot to break at 0% ee. This means that it is possible to determine simply and quantitatively the ee of a given primary amine sample **2** with unknown ee. That is, the host–pair **1** can be regarded as the host–pair reagent for the ee determination.

However, this host–pair reagent **1** cannot be applied to all kinds of chiral primary amine guests. It is important to know which type of guest is suitable for the ee determination. In general, when the IRIS value is ~1.5 or more (~0.65 or less), the guest is recommended for the ee determination, because a deep V-shaped plot (as shown in Fig. 4) can be used. An insufficient depth of a V-shaped plot will be interferred for precision of the ee determination. For example, in the case of the amino acid ester hydrochloride series, aspartic acid methyl ester hydrochloride and valine ethyl ester hydrochloride are possible to determine the ees because the IRIS values are obtained as 0.37 [with (S)-Asp-OMe^+] and 0.35 [with (S)-Val-OEt^+], respectively [13]. On the other hand, in the primary alkyl amine hydrochloride series, such as 1-phenylethyl amine and 1-(*p*-nitrophenyl)ethyl amine, the IRIS values are obtained as 0.95 and 1.03, respectively, so that these guests are not applicable for the ee determination [13]. Also, in the case of the secondary amine salt series, there exists no appropriate guest because of a much weaker complexation ability (and nearly unity IRIS value) [7].

The chiral recognition ability is strongly dependent on the structural combination between a given host and a given guest. The host–pair reagent (**1**) is

suitable for the ee determination of some amino acid ester guests. Another host–pair reagent is required for the ee determination of 1-phenylethyl amine etc. We are now attempting to develop a new host–pair reagent for this purpose.

5. Conclusions

In this article, we have clarified the following two fundamental features using chiral recognition FAB mass spectrometry. First, the IRIS value varies in a linear fashion with the ee quantity and produces a symmetric V-shaped plot. Thus, the EL–host method is potentially applicable for the ee determination of organic primary amine compounds. Second, the change in IRIS values measured by FAB mass spectra corresponds to the change in the concentration ratios of the diastereomeric complex ions formed in the matrix under the competitive equilibrium conditions. These findings show that chiral recogintion in solution can be successfully detected by the IRIS values (the FABMS/EL method), as far as the diastereomeric complex ions are concerned.

Acknowledgements

This work was partly supported by grants in aid for scientific research from the Ministry of Education, Science, Sports and Culture, Japan (Nos. 07454250 and 09874124) (M.S. and H.Y., respectively).

References

[1] D. Parker, Chem. Rev. 91 (1991) 1441.
[2] R.E. Gawley, J. Aubé, Principles of Asymmetric Synthesis, Pergamon, New York, 1996, Chap. 2, p. 45.
[3] Y. Okamoto, E. Yashima, Angew. Chem. Int. Ed. 37 (1998) 1020.
[4] E.L. Eliel, S.H. Wilen, Stereochemistry of Organic Compounds, Wiley, New York, 1994, p. 214.
[5] M.A. Baldwin, S.A. Howell, K.J. Welham, F.J. Winkler, Biomed. Environ. Mass Spectrom. 16 (1988) 357.
[6] Y.-N. Wu, Y.-P. Tu, Y.-J. Pan, Y.-Z. Chen, M. Cui, F.-R. Song, S.-Y. Liu, Anal. Lett. 30 (1997) 1399.
[7] M. Sawada, Y. Takai, H. Yamada, S. Hirayama, T. Kaneda, T. Tanaka, K. Kamada, T. Mizooku, S. Takeuchi, K. Uno, K. Hirose, T. Tobe, K. Naemura, J. Am. Chem. Soc. 117 (1995) 7726.
[8] M. Sawada, Mass Spectrom. Rev. 16 (1997) 73.
[9] M. Sawada, J. Mass Spectrom. Soc. Jpn. 45 (1997) 439.
[10] M. Sawada, Y. Takai, H. Yamada, J. Nishida, T. Kaneda, R. Arakawa, M. Okamoto, K. Hirose, T. Tanaka, K. Naemura, J. Chem. Soc., Perkin Trans. 2 (1998) 701.
[11] M. Sawada, M. Shizuma, Y. Takai, H. Adachi, T. Takeda, T. Uchiyama, Chem. Commun. (1998) 1453.
[12] X.X. Zhang, J.S. Bradshaw, R.M. Izatt, Chem. Rev. 97 (1997) 3313.
[13] M. Sawada, H. Yamaoka, Y. Kawai, H. Yamada, T. Azuma, T. Fujioka, T. Tanaka, J. Chem. Soc., Chem. Commun. (1998) 1569.
[14] R.A.W. Johnstone, M.E. Rose, Chem. Commun. (1983) 1268.
[15] R.A.W. Johnstone, I.A.S. Lewis, M.E. Rose, Tetrahedron 39 (1983) 1597.
[16] G.J. Langley, D.G. Hamilton, M.C. Grossel, J. Chem. Soc. Perkin Trans. 2 (1995) 929.
[17] R.D. Boss, A.I. Popov, Inorg. Chem 24 (1985) 3660.
[18] I.-H. Chu, V. Dearden, J.S. Bradshaw, P. Huszthy, R.M. Izatt, J. Am. Chem. Soc. 115 (1993) 4318.