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Analysis of enantiomeric excess using mass spectrometry: fast atom bombardment/sector and electrospray ionization/Fourier transform mass spectrometric approaches

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Abstract

The utility of fast atom bombardment (FAB) ionization on a sector mass spectrometer, and of electrospray ionization (ESI) on a Fourier transform ion cyclotron resonance mass spectrometer, for enantiomeric excess measurements was explored. Both methods involved the same host–guest system: (*R*,*R*)- or (*S*,*S*)-dimethyldiketopyridino-18-crown-6 (host) and α-(1-naphthyl)ethylammonium (guest). Both use an achiral amine (benzylamine for the FAB experiments, cyclohexylamine for the ESI experiments) as an internal reference compound and involve competitive complexation of the achiral and chiral amines with the chiral host. The FAB experiments are shown to give stable, reproducible results, but exhibit a smaller degree of enantiodiscrimination than the ESI experiments. The ESI experiments, which involve measurement of apparent guest exchange equilibrium constants, show a linear relationship between apparent equilibrium constant and enantiomeric excess. The apparent equilibrium constant is shown to be a composition-weighted average of the equilibrium constants for the two pure enantiomers. Enantiomeric impurities as small as about 2% can currently be detected. (Int J Mass Spectrom 185/186/187 (1999) 977–988)

Keywords: Enantiomer; Optical purity; Chiral recognition; Mass spectrometric determination

1. Introduction

Differences in the chirality of a compound may result in quite different chemical, physical, and physiological properties, so it is important to have rapid, accurate analytical techniques for determining enantiomeric excesses. Herein we report two rapid and sensitive mass spectrometric methods.

Various solution techniques have been employed to characterize enantiomeric excess. These include liquid membrane transport [1], solvent extraction [2–4], polarimetry [5,6], circular dichroism [6–8], calorimetry [9–11], nuclear magnetic resonance (NMR) [5,12–14], chromatography [15–17], and capillary electrophoresis [18]. However, these methods in general require relatively large amounts of sample. For example, liquid membrane transport, extraction, and polarimetric methods typically require a minimum of about 50 mg [1–3]. In the same host–guest chiral recognition system as we report herein, calo-

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Dedicated to Professor Michael T. Bowers on the occasion of his 60th birthday.

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rimetry generally required 20 mL of 0.1 or 0.01 M sample solution (100–1000 mg of host) [10], and extraction NMR consumed about 10 mg [12,13].

Even the well-established chiroptical methods, polarimetry and circular dichroism, have their disadvantages, and these limit their practical application. One disadvantage of polarimetry is that it requires relatively large sample sizes. In a typical host–guest chiral recognition experiment, 3.9 g of host and 3 g of guest were required for satisfactory results [5]. Also, the accuracy of polarimetry experiments is temperature- and solvent-dependent, so errors may arise from the concentration dependence of the specific rotation [6]. Circular dichroism consumes less sample: the concentration of the host and guest is about 0.10 mmol/L in a typical experiment [7,8]. Circular dichroism also has a good detection limit of $\sim$ 0.1 μg/mL, but it is considered to be too selective to be applied widely for practical analytical use, and it is not able to analyze racemic mixtures [6]. All the solution methods are of course subject to solvent effects, which can sometimes perturb the observed degree of enantiomeric discrimination [10]. In conclusion, the common feature of condensed phase methods is that they require relatively large sample sizes, and the experimental results may not reflect intrinsic chiral recognition properties because of solvent effects.

Although it is not inherently sensitive to absolute configuration, mass spectrometry offers an attractive alternative for the analysis of chiral compounds [19–30]. Gas phase studies provide results free of solvent effects, so the intrinsic factors contributing to the degree of chiral recognition of a chiral host can be determined. Perhaps a driving force behind the interest in mass spectrometry for chiral recognition studies is the sensitivity, speed, and simplicity of the technique, as well as the gas phase environment that only a mass spectrometer can provide.

Because enantiomers have identical molecular masses, mass spectrometric methods for determining enantiomeric excess generally rely on differences in the reactivity of the enantiomers with a chiral reference compound. The degree of chiral recognition is usually determined by measuring the relative peak intensity ratio (RPI) or the apparent free energy difference between reactions involving the two enantiomers.

Several mass spectrometric methods have been used in chiral recognition studies. These include chemical ionization (CI) mass spectrometry [24,31], fast atom bombardment (FAB) double focusing mass spectrometry [19–22,25,32], and electron impact (EI) and electrospray ionization (ESI) Fourier transform ion cyclotron resonance mass spectrometry (FTICR/MS) [26,27]. Both the CI and EI techniques require that the samples have significant vapor pressure. Sometimes heating of the sample probe is necessary, but this may degrade or decompose the sample, and also make the temperature determination difficult. These ionization methods become impractical with larger, less volatile samples.

Application of fast atom bombardment ionization methods to chiral recognition makes the study of bigger chiral molecules feasible; this area has recently been reviewed [32]. In a typical FAB experiment, a known ratio of chiral analyte, chiral reference molecule, and achiral reference molecule is mixed with a matrix, typically either nitrobenzyl alcohol or glycerol. A few microliters of the mixture is deposited on the FAB tip and bombarded by fast-moving atoms. Generally, adducts of the analyte with the chiral and achiral reference compounds are observed. The intensity ratio of the chiral adduct to the achiral adduct has been shown to remain stable over a sufficient time period to characterize the sample [19–22]. The measurement is then repeated with the other enantiomer. The degree of chiral recognition is defined by the relative peak intensity ratio of the enantiomer pairs from the two sets of spectra. Alternatively, instead of using an achiral reference, a racemic mixture may be used with one of the enantiomers isotopically labeled [33–35]. The difference in the peak intensities for the labeled and unlabeled diastereomers yields the degree of recognition directly.

The FAB method is simple and fast, and is considered to be a good method for rapid screening of chiral compounds. It has also been applied to the measurement of enantiomeric excesses [36,37]. However, it is not clear whether chiral recognition occurs in the solution mixture, in the selvedge region, or in

the gas phase, or if the observed recognition arises from reactions in all three regions. It is therefore hard to determine what conditions are responsible for the observed results. The chemistry in the FAB matrix is a complicated process [38,39], and matrix effects may also affect the results of chiral recognition experiments. Comparing the same host–guest systems, the degree of chiral recognition measured using FABMS is much lower than that observed using Fourier transform ion cyclotron resonance mass spectrometry [22,26,27], and it is even lower than the results from solution experiments [22]. Further, equilibrium is probably not reached in the FAB experiment, so the thermodynamic parameters that characterize the chiral recognition process are difficult to obtain.

ESI offers another route to the mass spectrometry of chiral compounds. First described by Fenn et al. [40,41], ESI has shown remarkable ability for ionizing large molecules. This is important because many of the molecules of interest are large and involatile. Further, ESI has been interfaced with both liquid chromatography [42] and capillary electrophoresis mass spectrometry [43], suggesting powerful techniques that combine the proven ability of chiral separation methods with the sensitivity of mass spectrometric detection. Our recent work demonstrated the use of ESI in combination with ion/molecule chemistry as a mass spectrometric method to distinguish between enantiomers and characterize the thermochemistry of their reactions [27]. Herein we extend that work to the determination of enantiomeric excesses in mixtures. We also report similar experiments using fast atom bombardment–double focusing mass spectrometry, and compare and contrast the two techniques.

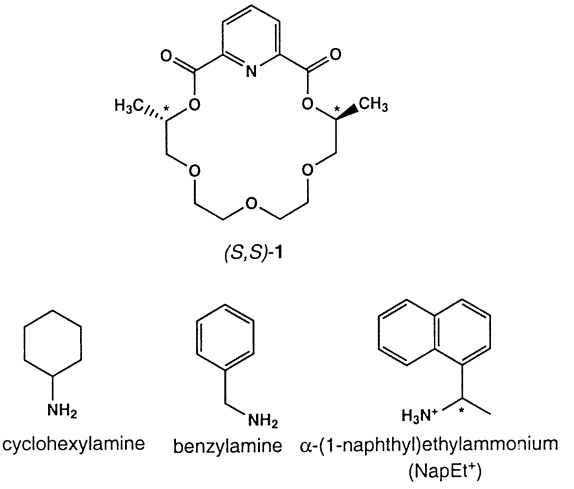

Fig. 1. Structures and abbreviations for host and guest molecules used in this study. Only the (*S*,*S*)-enantiomer of the host is shown.

2. Experimental

2.1. Setups for chiral mixture analysis

The FTICR/MS experiments employed a Bruker Apex 47e Fourier transform ion cyclotron resonance mass spectrometer, which has been described elsewhere [27]. In brief, the instrument features a 4.7 T superconducting magnet and an external ion source. The pressure is maintained at a base vacuum of about 1×10^{-9} mbar by five stages of differential pumping. Ions are generated using a commercial electrospray source (Analytica, Branford, CT, and Bruker Daltonics, Billerica, MA) that we have modified for microspray with a 50 μm fused silica capillary spray tip. Typically, 1.3 kV is applied to a zero-dead-volume union and is transmitted through the spray electrolyte to the capillary tip. Typically, the electrospray flow rate is 10 μL/h, maintained using a syringe pump (Harvard Apparatus). The spray is directed into a heated stainless steel capillary drying tube, maintained near ground with respect to the spray tip. The gas flow exiting the drying tube is skimmed, and ions in the flow are transported to a hexapole ion trapping device and accumulated (typically for 0.1 s), and then transferred to the trapping cell via electrostatic focusing. The trapping cell was at ambient temperature (about 300 K in all experiments).

Structures of the host and guest species used in these experiments are given in Fig. 1. Both enantiomers of the host molecule, dimethyldiketopyridino-18-crown-6 [referred to as (*R*,*R*)-**1** and (*S*,*S*)-**1** herein] were electrosprayed to generate protonated ions. The synthesis of this chiral host has been described

[44]. *S*-α-(1-naphthyl)ethylamine (purity > 99%, referred to as *S*-NapEt herein) was purchased from Fluka, and cyclohexylamine (purity > 97.9%) was purchased from Fisher. All chemicals were used as supplied, with the exception that they were purified through several freeze–pump–thaw cycles prior to being introduced into the vacuum system.

Experimental procedures similar to those used here have been described [27]. We detail only features that are unique to the current experiments. Typically, chiral and achiral reference compounds (*S*-NapEt and cyclohexylamine, respectively) were introduced into the instrument through precision variable leak valves (Varian, Palo Alto, CA). Care was taken to ensure that the partial pressure of the first amine introduced was stable before introducing the second. Thereafter, we assumed the partial pressure of each amine remained constant during the experiment. The pressures were measured using a cold cathode ionization gauge (Balzers). Measurement of absolute pressure is not necessary because the results are not directly related to the absolute pressure reading; only the pressure ratio of the two amines is required. Typically, the partial pressure of each amine was about 5×10^{-8} mbar. Mixtures of known amounts of the enantiomers of (*R*,*R*)-**1** and (*S*,*S*)-**1** were prepared in 80:18:2 methanol:water:acetic acid and electrosprayed to generate protonated chiral crowns. The total concentration of each mixture (the sum for the two enantiomers) was 0.1 mg/mL. The protonated chiral crowns, trapped by the magnetic and electrostatic fields of the instrument, were allowed to react with *S*-NapEt and cyclohexylamine in the trapping cell to form adducts. One of these adducts was then isolated and allowed to react with the neutral amines. In effect, isolation perturbs the system away from an equilibrium population, and subsequent reaction results in restoration of equilibrium. Because the host enantiomers have identical mass, the observed intensity of the adduct peak is contributed by both enantiomers. The apparent reaction in the cell is as follows, where "[(*R*,*R*)-**1** + (*S*,*S*)-**1**]" refers to the mixture of host enantiomers and "Ref" refers to the achiral reference amine (cyclohexylamine in this case).

$$[(R,R)\text{-}\mathbf{1} + (S,S)\text{-}\mathbf{1}]\mathrm{H}^+ \cdot \mathrm{Ref} + S\text{-NapEt} \underset{}{\overset{K_{app}}{\rightleftharpoons}} [(R,R)\text{-}\mathbf{1} + (S,S)\text{-}\mathbf{1}]\mathrm{H}^+ \cdot S\text{-NapEt} + \mathrm{Ref} \quad \text{(R1)}$$

The apparent equilibrium constant for Reaction (1) is given in Eq. (1), where *P* represents the partial pressure of either the achiral reference compound or the chiral reference, *S*-NapEt, and *I* is the observed mass spectrometric signal intensity (typically from the peak amplitude) of one of the host enantiomers with either the achiral or chiral reference, as indicated. Eq. (1) reflects the fact that the observed intensities of the mass spectral peaks from the complexes arise from mixtures of (*R*,*R*)-**1** and (*S*,*S*)-**1**, which are identical in mass:

$$K_{app} = \frac{(I_{R,R\cdot S} + I_{S,S\cdot S})\,P_{Ref}}{(I_{R,R\cdot Ref} + I_{S,S\cdot Ref})\,P_{S\text{-NapEt}}} \quad (1)$$

Each reaction was monitored as a function of time in both "forward" and "reverse" directions, as has been described previously [26,27]. The ion intensity ratio of chiral complex to achiral complex should be the same in both directions if equilibrium is reached. Characterization of the chiral mixtures hinges on the fact that the thermodynamic equilibrium constants differ for the diastereomeric (*R*,*R*)-**1** · *S*-NapEt and (*S*,*S*)-**1** · *S*-NapEt complexes. Chiral mixtures having different (*R*,*R*)-**1** and (*S*,*S*)-**1** excesses should have different apparent equilibrium constants, as will be shown.

2.2. FABMS experiments

The FABMS experiments employed a Jeol JMS-SX102A double focusing mass spectrometer. The instrument was equipped with a Jeol MS-FAB 10 gun, and the source pressure was typically about 5×10^{-6} Torr. (*S*,*S*)-**1** was used as chiral host. The FAB matrix was nitrobenzyl alcohol (purity >98%), purchased from Aldrich and used as supplied. The perchlorate salts of *R*- and *S*-NapEt and benzylamine were prepared as described [12]. The ammonium salts were dissolved in methanol (HPLC grade, Mallinckrodt), and the concentrations of *R*-NapEt, *S*-NapEt, and benzylamine were 0.10, 0.10, and 0.11 M, respec-

tively. The chiral host was dissolved in 80:17:3 methanol:water:acetic acid with a concentration of 0.014 M (5 mg/mL).

In a typical FABMS experiment, the sample solution was prepared by mixing the following solutions: 3 μL of 0.014 M (*S*,*S*)-**1**, 5 μL of 0.10 M *S*-NapEt, 5 μL of 0.11 M benzylamine, and 13 μL of nitrobenzyl alcohol (matrix). The mixture was thoroughly mixed using an ultrasonic vibrator and allowed to equilibrate for at least 24 h. The double focusing mass spectrometer was operated at an accelerating voltage of 10 kV with a mass range of 220–1000 u. Xenon was used as the fast atom beam, accelerated to 3 kV with an emission current of 20 mA. Scans were collected for 10 min (20 min in some experiments) with a scan rate of 10 s/decade. About 15 scans were averaged to obtain the intensity ratio for each enantiomer in a single run. The final RPI value is the average of several runs. Once the spectra for one guest enantiomer (*S*-NapEt, for example) were collected, the experiment was quickly switched to the other guest enantiomer (*R*-NapEt), thus minimizing the effects of instrument fluctuations.

3. Results

3.1. Enantiomeric excess determination using ESI-FTICR/MS

Fig. 2 shows typical equilibrium mass spectra at different enantiomeric excesses. The peak at mass 453 is the complex of the chiral host mixture with the reference achiral amine (cyclohexylamine), whereas mass 525 is the complex of the chiral host mixture with the chiral amine, *S*-NapEt. From the spectra shown in Fig. 2, it is clear that the chiral guest adduct/achiral guest adduct ratio increases as the (*S*,*S*)-**1** fraction of the host mixture decreases. This is consistent with what we observed in our previous experiments [26,27]; that is, (*R*,*R*)-**1** binds *S*-NapEt more favorably than does (*S*,*S*)-**1**. Thus, as the fraction of weaker binding (*S*,*S*)-**1** in the mixture increases, the achiral cyclohexylamine competes increasingly more effectively for the host.

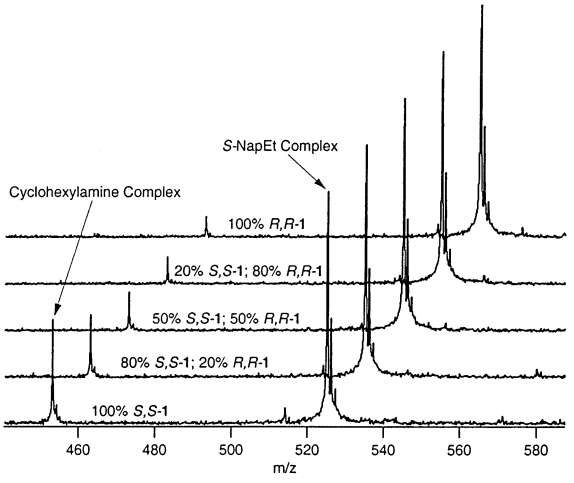

Fig. 2. FTICR mass spectra, at guest exchange equilibrium, for electrosprayed hosts with various enantiomeric excesses. The total host concentration in each experiment was 0.1 mg/mL. The guests were cyclohexylamine (which served as an achiral reference) and (*S*)-(α-1-naphthyl)ethylamine (which served as chiral discriminator). All the spectra are scaled to the height of the (*S*)-(α-1-naphthyl)ethylamine complex peak.

The enantiomeric excess in the chiral mixtures can be characterized in terms of apparent equilibrium constant K_{app}. Fig. 3 shows the linear relationship between measured apparent equilibrium constant K_{app} and (*R*,*R*)-**1** fraction in the mixture. Least-squares fitting gives a correlation coefficient of 0.9989 and a slope of 4.06 ± 0.11% *ee*. Because the apparent equilibrium ratios can typically be measured to within better than 0.5, this suggests that potentially enantiomeric excesses should be measurable to within about 2% using this technique.

3.2. Enantiomeric excess determination using FABMS

Amine exchange equilibrium was used in the FABMS experiments. Fig. 4 includes the mass spectra obtained for the reactions of (*S*,*S*)-**1** with *R*-NapEt and (*S*,*S*)-**1** with *S*-NapEt. The peaks at mass 354, 461, and 525 correspond to chiral host, chiral host-benzylamine adduct, and chiral host-chiral amine adduct, respectively. Other peaks are either matrix peaks or trace impurities. The results are summarized

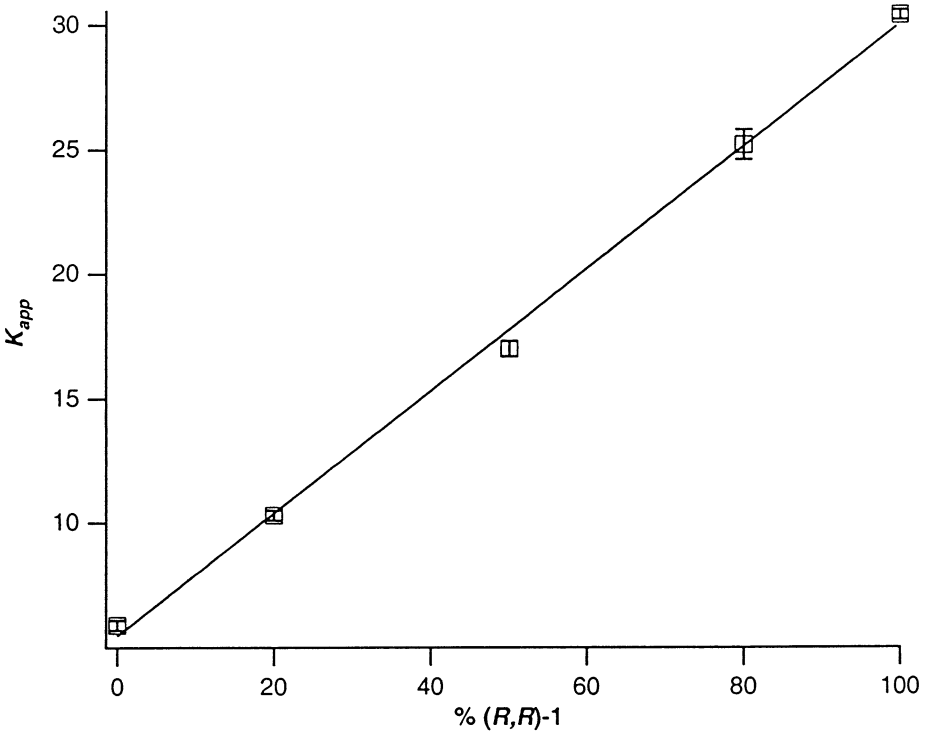

Fig. 3. Apparent equilibrium constant K_{app} as a function of host enantiomeric makeup. Total host concentration in the electrospray solution was 0.1 mg/mL. Error bars represent 1 standard deviation from replicate runs. Other conditions as in Fig. 2.

in Table 1. The RPI of chiral adduct to achiral adduct is stable over long experiment times, and is insensitive to the guest/host ratio in the matrix as long as the guest is present in large excess. Shown in Fig. 5 is the relative peak intensity ratio of chiral adduct to achiral adduct over a 10 min experiment period. The RPI value is frequently stable over the course of a 20 min experiment. In practical applications, less than 5 min should be enough to obtain reliable data. The degree of chiral recognition observed here using amine exchange is much larger than that reported using a host exchange system [22], wherein a stability constant ratio of 1.17 was found, compared with the RPI ratio of 1.83 observed in our experiment. The results from both ESI-FTICR/MS and FABMS are in agreement. Both methods suggest the preference of heterochiral complex formation; that is, (*R*,*R*)-**1** binds *S*-NapEt more strongly than does (*S*,*S*)-**1**, and vice versa.

4. Discussion

4.1. Mathematical description of ESI-FTICR results

Electrospray ionization is a concentration-dependent process. The characteristics of the electrosprayed solution, including analyte concentration, conductivity, solvent, and pH, and the electrospray parameters, such as solution flow rate, and voltages applied on the spray capillary and interface, may affect the observed ion signal intensity [45–49]. Other factors, such as the transfer rate of ions from droplets to the gas phase, the efficiency of conversion of droplet charge to gas phase ions, and the ion transmission efficiency of the ion transport system have also been considered. A comprehensive mathematical model has been proposed to consider most of the above mentioned factors [48,49]. Experimental data in the low concentration range (10^{-8}–5×10^{-6} M) are in agreement with

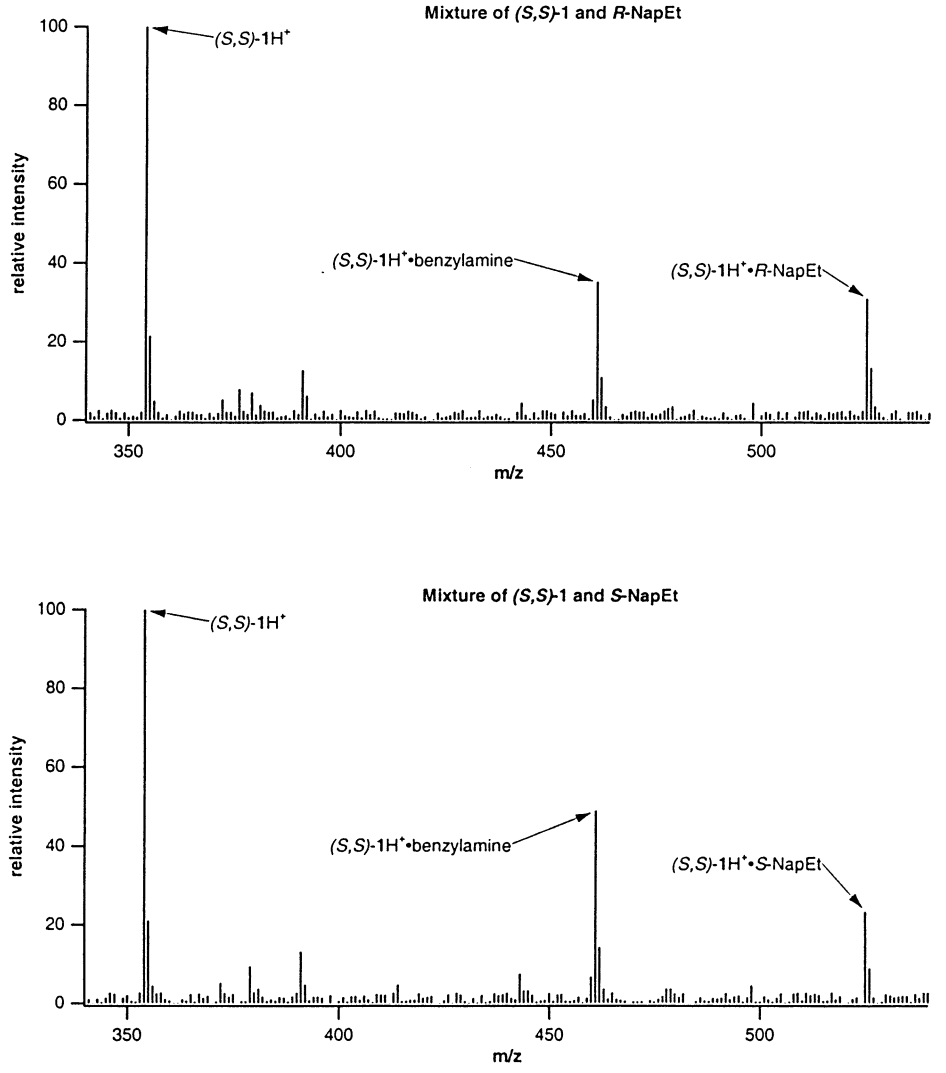

Fig. 4. FAB mass spectra (average of 16 scans) for a mixture of chiral host [(*S*,*S*)-**1**], chiral amine, and benzylamine. Top frame: chiral amine = *R*-NapEt, ratio of chiral adduct/achiral adduct = 0.86 ± 0.01. Bottom frame: chiral amine = *S*-NapEt, ratio of chiral adduct/achiral adduct = 0.46 ± 0.01.

those predicted using the model. The model also provides valuable predictions in the high concentration range (10^{-5}–10^{-2} M). However, the electrospray ionization process is complicated, and its mechanism is not completely understood.

The situation in our experiment is simpler, because the ions of interest are identical in mass, similar in size, and exist in the same environment. They differ only in absolute configuration, and therefore can be distinguished only in a chiral environment. The two enantiomeric ions also have the same ionization and transport experience until they are trapped in the cell. Therefore we can assume all the factors determining the observed ion intensity are the same except the interaction with the chiral probe molecule in the trapping cell.

The linearity of the data in Fig. 3 suggests a simple relationship between the apparent equilibrium con-

Table 1
Comparison of observed degree of preference for *R*-NapEt by (*S*,*S*)-**1** by FAB-sector and ESI-FTICR methods. Results obtained in methanol included for comparison

FAB sector [a]		ESI-FTICR [b]	Methanol solution [c]
Guest/host ratio	RPI_R/RPI_S		
8	1.83 ± 0.05		
15	2.02 ± 0.07		
25	1.90 ± 0.07		
37	1.95 ± 0.12		
		3.3 ± 0.4	2.6

[a] Average and standard deviation over 10 scans.
[b] Ratio of *R*-NapEt · (*S*,*S*)-**1** to *S*-NapEt · (*S*,*S*)-**1** at equilibrium, obtained by using cyclohexylamine as an achiral reference.
[c] Equilibrium ratio of *R*-NapEt · (*S*,*S*)-**1** to *S*-NapEt · (*S*,*S*)-**1**, from solution equilibrium constant data in [54].

stant K_{app} and enantiomeric excess. We now proceed to derive an expression for the relationship. As was noted in Reaction (1) and Eq. (1), the observed signal arises from a mixture of the two enantiomers. Equilibrium constants for the optically pure enantiomers K_{RR} and K_{SS} are known [27], or (better for an analytical determination) can be determined under the same conditions as are used for the analyte. Expressions for K_{RR} and K_{SS} in terms of mass spectrometric signal intensities I and neutral gas pressures P are given in Eqs. (2) and (3):

$$K_{RR} = \frac{I_{R,R\cdot S}P_{\mathrm{Ref}}}{I_{R,R\cdot \mathrm{Ref}}P_{S\text{-NapEt}}} \tag{2}$$

$$K_{SS} = \frac{I_{S,S\cdot S}P_{\mathrm{Ref}}}{I_{S,S\cdot \mathrm{Ref}}P_{S\text{-NapEt}}} \tag{3}$$

Solving each of these for $I_{R,R\cdot S}$ and $I_{S,S\cdot S}$, respectively, and substituting into Eq. (1) yields Eq. (4).

$$K_{\mathrm{app}} = \frac{(K_{RR}I_{R,R\cdot \mathrm{Ref}} + K_{SS}I_{S,S\cdot \mathrm{Ref}})}{(I_{R,R\cdot \mathrm{Ref}} + I_{S,S\cdot \mathrm{Ref}})} \tag{4}$$

We note that both $I_{R,R\cdot \mathrm{Ref}}$ and $I_{S,S\cdot \mathrm{Ref}}$ are proportional to the concentrations of the respective host molecules in the spray solution. The proportionality constant comprises contributions from electrospray efficiency, ion transport, pressure of neutral, achiral reference compound, etc., but it is important to note that the value of the proportionality constant is the same for both hosts because they are enantiomers in an achiral environment. Therefore, if C_{RR} and C_{SS} represent the concentrations of the host enantiomers in solution, Eq. (4) can be rewritten such that the proportionality constant cancels:

$$K_{\mathrm{app}} = \frac{(K_{RR}C_{R,R} + K_{SS}C_{S,S})}{(C_{R,R} + C_{S,S})} \tag{5}$$

Let α_{RR} be the fraction of the host molecule that is the *R*,*R* enantiomer, and α_{SS} be the fraction that is the *S*,*S* enantiomer. It is easy to see that Eq. (5) expresses the simple idea that K_{app} is just the composition-weighted average of the equilibrium constants for the two pure enantiomers [Eq. (6)]:

$$K_{\mathrm{app}} = K_{RR}\alpha_{RR} + K_{SS}\alpha_{SS}. \tag{6}$$

Substituting into Eq. (6) and solving for α_{RR}, we obtain the desired expression, Eq. (7):

$$\alpha_{RR} = \frac{K_{\mathrm{app}} - K_{SS}}{K_{RR} - K_{SS}} \tag{7}$$

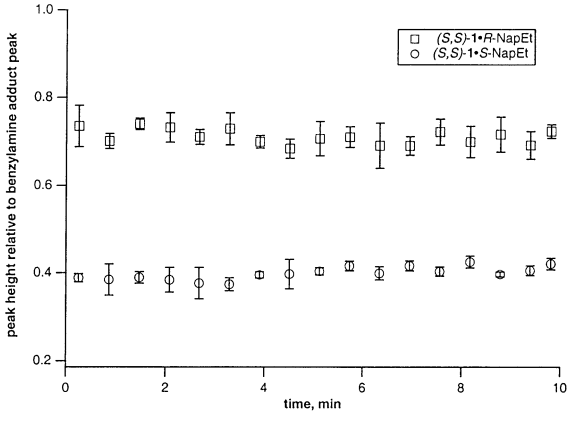

Fig. 5. Behavior of chiral adduct/achiral adduct ratio from FAB mass spectra as a function of time. The guest (*R*- or *S*-NapEt) was present in the FAB matrix in a 25-fold excess over the host, (*S*,*S*)-**1**. Error bars represent 1 standard deviation from a set of replicate scans.

4.2. Analytical utility of the ESI-FTICR method

An ideal analytical method for the determination of enantiomeric excess should require very small samples, be rapidly and easily performed, be generally applicable to a wide variety of samples, and be sensitive to small enantiomeric impurities. The ESI-FTICR technique does very well with the first of these criteria, reasonably well with the second and third, and is satisfactory in the fourth.

The sample requirements for micro-electrospray are very small. With a typical syringe flow rate of 10 μL/h, less than 5 μL of sample is enough for the experiment. Concentrations in the micromolar range are generally sufficient for electrospray, so conservatively, the total sample consumed is on the order of a picomole. Because attomole sensitivity has been demonstrated for ESI [50,44], it is likely that optimization of the method could lead to significant improvements. Even without optimization, it is clear that our experimental method is at least 100 times more sensitive (in terms of sample consumption) than the solution methods [1–3,10].

This type of determination can be carried out fairly rapidly and easily. While ESI-FTICR clearly cannot compete with optical spectroscopic methods for ease of use, it is still a relatively straightforward technique. Once the background amine pressures are stable, a large number of unknown mixtures can be sprayed and characterized. Approximately 10–20 min are required to manually set up and carry out each measurement, but automation could decrease this time considerably. The sample preparation requirements are the same as for any ESI measurement.

The breadth of the equilibrium techniques remains to be established. The use of electrospray ionization eliminates the requirement of sample volatility, although it is still essential that the reference compounds have significant vapor pressures. It is certain that only a limited range of host–guest combinations will be amenable for enantiomeric excess determinations. For example, the dimethyldiketopyridino-18-crown-6 host used in these experiments exhibits excellent enantiodiscrimination for α-(1-napthyl)ethylamine, but is very poor at distinguishing between the enantiomers of 2-butylamine [27]; it appears from the earlier work that this host best recognizes guests capable of good face-to-face π-stacking interactions with the host pyridino moeity. If this technique is to gain more than purely academic application, it will be necessary to identify a broader range of host–guest combinations exhibiting good enantiodiscrimination.

As was noted above, dimethyldiketopyridino-18-crown-6 is capable of detecting NapEt enantiomeric impurities down to about the 2% level. Thus, ESI-FTICR/MS is not as effective as chromatographic techniques, which can detect impurities of 1% or less [16,17], but its performance is satisfactory, especially when limited amounts of material are available for analysis. The NapEt guest represents a favorable case, because enantiodiscrimination for this guest is strong. Eq. (7) shows that the difference between equilibrium constants for enantiomers is critical in determining the minimum measurable enantiomeric excess. Thus, as with the issue of breadth, identification of systems exhibiting strong enantiodiscrimination is essential to the measurement of small enantiomeric impurities.

4.3. Analytical utility of the FAB-sector method

The FAB-sector techniques can be judged using the same criteria developed above. Although it is not as sensitive as ESI-FTICR, the FAB-sector method also has modest sample requirements. The FAB experiments typically require host–guest complex concentrations of about 0.1 M, with a total sample size of about 1 μL, so about 10^{-7} mol of sample are needed for the measurement. The FAB-sector method excels in ease of use and speed. Sample preparation is simple, and measurements require only about 5 min. Although we have not explored the issue directly, the generality of this method is probably comparable to that of ESI-FTICR, with the caveat that the observed enantiodiscrimination is less for the FAB-sector technique. In addition, the molecular weight range that can be addressed using FAB is smaller than that of ESI. Finally, because the degree of observed enantiodiscrimination is less for the FAB-sector technique, this method might be expected to be less capable of detecting enantiomeric impurities than ESI-FTICR.

However, we expect this will be offset by the much higher dynamic range typically achievable with the ion detectors normally used with sector mass analyzers.

4.4. Molecular recognition and ion formation in FAB

The nature of the FAB process [39,51] leads to ambiguities about where the ions form, and where the reactions occur that lead to molecular recognition. In particular, it is unclear whether recognition originates from reactions in solution, in the selvedge region, or in the gas phase. It is possible that all three environments play a role [39,52]. Despite the ambiguity about its exact mechanism, study of the application of FABMS to chiral recognition has been extensive [32,53]. Although the FAB matrix clearly affects ion intensity, when FAB is applied to chiral recognition matrix effects should be minimal, because the target molecules are chiral, and the enantiomers show the same reactivity toward an achiral matrix. The RPI method essentially compares two reactions (involving the two enantiomers), assuming the solution environment and experimental conditions are the same for both enantiomers. Comparison of the FAB results with the degree of molecular recognition observed in the gas phase and in solution may give some insight into the type of environment that leads to the ions observed in FAB.

Fenselau et al. [39] have pointed out that no matter what mechanism dominates the FAB ion formation process, most researchers prefer preformed ions in solution as a useful and predictive model. Based on comparison with our FTICR/MS results, which clearly come from gas phase processes, we believe enantiomeric discrimination in FAB occurs primarily in solution, with possible contributions from other regions. The degree of recognition in FABMS is much smaller than that in the gas phase, as is shown in Table 1, and is a little smaller than in methanol solution (the observed hetero/homo preference is 1.9 in FABMS and 2.6 in methanol solution). Although these results are far from conclusive, they suggest that the ion abundances observed in the FAB spectrum more closely resemble solution than gas phase conditions.

5. Conclusions

Guest exchange equilibrium involving chiral hosts can be applied to the analytical determination of enantiomeric excess, and the method is general enough that it can be used either with electrosprayed ions analyzed using FTICR/MS or with FAB-generated ions probed using a sector instrument. In the FTICR, where the system is clearly at equilibrium, the observed equilibrium constant is a composition-weighted average of the equilibrium constants seen for the pure enantiomers.

Comparing the two mass spectrometric methods used in this article, it is clear that experiments can be performed with smaller samples using ESI-FTICR/MS than using FAB. The former method also unambiguously reflects gas phase equilibrium conditions, so interpretation of the results in terms of fundamental thermodynamic properties is straightforward. On the other hand, with ESI-FTICR/MS it is essential to take great care to ensure that the system is truly at equilibrium, necessitating longer experiment times than are needed for the FAB runs. In addition, it has been our experience that sample preparation is simpler and quicker for the FAB experiments.

While the amount of sample required for mass spectrometric determination of enantiomeric excess is very small (especially for ESI-FTICR/MS), a number of difficulties need to be successfully addressed before these methods can become generally practical. The need to perform measurements on both pure enantiomers as well as on the analyte is an obvious disadvantage. Neither mass spectrometric method is as sensitive to small enantiomeric impurities as, for example, chromatographic techniques. Most importantly, systems that have a high intrinsic degree of enantiomeric discrimination need to be identified and characterized. This in turn requires a better understanding of the fundamental factors that lead to chiral recognition.

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References

[1] M. Newcomb, J.L. Toner, R.C. Helgeson, D.J. Cram, J. Am. Chem. Soc. 101 (1979) 4941.
[2] G.W. Gokel, J.M. Timko, D.J. Cram, J. Chem. Soc., Chem. Commun. (1975) 394.
[3] D.S. Lingenfelter, R.C. Helgeson, D.J. Cram, J. Org. Chem. 46 (1981) 393.
[4] W. Bussmann, J.-M. Lehn, U. Oesch, P. Plumere, W. Simon, Helv. Chim. Acta 64 (1981) 657.
[5] E.P. Kyba, J.M. Timko, L.J. Kaplan, F.D. Jong, G.W. Gokel, D.J. Cram, J. Am. Chem. Soc. 100 (1978) 4555.
[6] P. Schreier, A. Bernreuther, M. Huffer, Analysis of Chiral Organic Molecules—Methodology and Applications, Walter de Gruyter, New York, 1995.
[7] T. Morozumi, S. Shinkai, Chem. Lett. (1994) 1515.
[8] T. Morozumi, S. Shinkai, J. Chem. Soc., Chem. Commun. (1994) 1219.
[9] J.D. Lamb, J.E. King, J.J. Christensen, R.M. Izatt, Anal. Chem. 53 (1981) 2127.
[10] R.M. Izatt, X.X. Zhang, P. Huszthy, C.Y. Zhu, J.K. Hathaway, T. Wang, J.S. Bradshaw, J. Inclusion Phenom. Mol. Recognit. Chem. 18 (1994) 353.
[11] R.M. Izatt, R.E. Terry, D.P. Nelson, Y. Chan, D.J. Eatough, J.S. Bradshaw, L.D. Hansen, J.J. Christensen, J. Am. Chem. Soc. 98 (1976) 7626.
[12] R.B. Davidson, J.S. Bradshaw, B.A. Jones, N.K. Dalley, J.J. Christensen, R.M. Izatt, F.G. Morin, D.M. Grant, J. Org. Chem. 49 (1984) 353.
[13] P. Huszthy, J.S. Bradshaw, C.Y. Zhu, R.M. Izatt, S. Lifson, J. Org. Chem. 56 (1991) 3330.
[14] Y. Li, L. Echegoyen, M.V. Martinez-Diaz, J.D. Mendoza, T. Torres, J. Org. Chem. 56 (1991) 4193.
[15] G.D.Y. Sogah, D.J. Cram, J. Am. Chem. Soc. 101 (1979) 3035.
[16] S. Ahuja, Chiral Separations by Liquid Chromatography, American Chemical Society, Washington, DC, 1991.
[17] L.R. Sousa, G.D.Y. Sogah, D.H. Hoffman, D.J. Cram, J. Am. Chem. Soc. 100 (1978) 4569.
[18] R. Kuhn, F. Erni, T. Bereuter, J. Hausler, Anal. Chem. 64 (1992) 2815.
[19] M. Sawada, Y. Takai, H. Yamada, S. Hirayama, T. Kaneda, T. Tanaka, K. Kamada, T. Mizooku, S. Takeuchi, K. Ueno, K. Hirose, Y. Tobe, K. Naemura, J. Am. Chem. Soc. 117 (1995) 7726.
[20] M. Sawada, Y. Okumura, M. Shizuma, Y. Takai, Y. Hidaka, H. Yamada, T. Tanaka, T. Kaneda, K. Hirose, S. Misumi, S. Takahashi, J. Am. Chem. Soc. 115 (1993) 7381.
[21] M. Sawada, Y. Okumura, H. Yamada, Y. Takai, S. Takahashi, T. Kaneda, K. Hirose, S. Misumi, Org. Mass Spectrom. 28 (1993) 1525.
[22] G. Pócsfalvi, M. Lipták, P. Huszthy, J.S. Bradshaw, R.M. Izatt, K. Vékey, Anal. Chem. 68 (1996) 792.
[23] E.N. Nikolaev, G.T. Goginashvili, V.L. Tal'rose, R.G. Kostyanovsky, Int. J. Mass Spectrom. Ion Processes 86 (1988) 249.
[24] N.M. Sellier, C.T. Bouillet, D.L. Douay, J.-C.E. Tabet, Rapid Commun. Mass Spectrom. 8 (1994) 891.
[25] T.T. Dang, S.F. Pedersen, J.A. Leary, J. Am. Soc. Mass Spectrom. 5 (1994) 452.
[26] I.-H. Chu, D.V. Dearden, J.S. Bradshaw, P. Huszthy, R.M. Izatt, J. Am. Chem. Soc. 115 (1993) 4318.
[27] D.V. Dearden, C. Dejsupa, Y. Liang, J.S. Bradshaw, R.M. Izatt, J. Am. Chem. Soc. 119 (1997) 353.
[28] M.I. Nikolaeva, E.N. Nikolaev, D.P. Ridge, J.H. Futrell, 44th ASMS Conference on Mass Spectrometry and Allied Topics, Portland, OR, 1996, p. 1209.
[29] E.N. Nikolaev, E.V. Denisov, 44th ASMS Conference on Mass Spectrometry and Allied Topics, Portland, OR, 1996, p. 1210.
[30] E.V. Denisov, V. Shustryakov, E.N. Nikolaev, F.J. Winkler, R. Medina, 44th ASMS Conference on Mass Spectrometry and Allied Topics, Portland, OR, 1996, p. 1414.
[31] J. Martens, S. Lübben, W. Schwarting, Z. Naturforsch. 43b (1991) 320.
[32] M. Sawada, Mass Spectrom. Rev. 16 (1997) 73.
[33] H.M. Fales, G.J. Wright, J. Am. Chem. Soc. 99 (1977) 2339.
[34] D.H. Williams, C. Bradley, G. Bojesen, S. Shantikarn, L.C.E. Taylor, J. Am. Chem. Soc. 103 (1981) 5700.
[35] M. Sawada, Y. Takai, H. Yamada, T. Kaneda, K. Kamada, T. Mizooku, K. Hirose, Y. Tobe, K. Naemura, J. Chem. Soc., Chem. Commun. (1994) 2497.
[36] M.A. Baldwin, S.A. Howell, K.J. Welham, F.J. Winkler, Biomed. Environ. Mass Spectrom. 16 (1988) 357.
[37] 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.
[38] K. Vékey, L.F. Zerilli, Org. Mass Spectrom. 26 (1991) 939.
[39] C. Fenselau, R.J. Cotter, Chem. Rev. 87 (1987) 501.
[40] M. Yamashita, J.B. Fenn, J. Phys. Chem. 88 (1984) 4451.
[41] M. Yamashita, J.B. Fenn, J. Phys. Chem. 88 (1984) 4671.
[42] J.F.J. Banks, J.P. Quinn, C.M. Whitehouse, Anal. Chem. 66 (1994) 3688.
[43] S.A. Hofstadler, F.D. Swanek, D.C. Gaie, A.G. Ewing, R.D. Smith, Anal. Chem. 67 (1995) 1477.
[44] B.A. Jones, J.S. Bradshaw, R.M. Izatt, J. Heterocycl. Chem. 19 (1982) 551.
[45] A.T. Blades, M.G. Ikonomou, P. Kebarle, Anal. Chem. 63 (1991) 2109.
[46] P. Kebarle, L. Tang, Anal. Chem. 65 (1993) 972A.
[47] M.G. Ikonomou, A.T. Blades, P. Kebarle, Anal. Chem. 63 (1991) 1989.
[48] L. Tang, P. Kebarle, Anal. Chem. 63 (1991) 2709.
[49] L. Tang, P. Kebarle, Anal. Chem. 65 (1993) 3654.
[50] G.A. Valaskovic, N.L. Kelleher, D.P. Little, D.J. Aaserud, F.W. McLafferty, Anal. Chem. 67 (1995) 3802.

[51] G. Székely, J. Allison, J. Am. Soc. Mass Spectrom. 8 (1997) 337.
[52] C. Fenselau, Fast Atom Bombardment, in A. Benninghoven (Ed.), Ion Formation from Organic Solids, Springer, Berlin, 1983, p. 90.
[53] A. Dobó, M. Lipták, P. Huszthy, K. Vékey, Rapid Commun. Mass Spectrom. 11 (1997) 889.
[54] R.M. Izatt, T. Wang, J.K. Hathaway, X.X. Zhang, J.C. Curtis, J.S. Bradshaw, C.Y. Zhu, J. Inclusion Phenom. Mol. Recogn. Chem. 17 (1994) 157.