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Enantioselective gas-phase ion–molecule reactions in a quadrupole ion trap

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Abstract

Ion trap mass spectrometry (ITMS) was employed to study chiral guest-exchange reactions in gas-phase complexes of amino acids and β-cyclodextrin. Enantioselectivity was obtained with a selected group of amino acids containing alkyl side chains. The selectivity for the amino acids increased with the size of the side chain except for Phe where the selectivity dropped off considerably. The results were consistent with those obtained earlier with Fourier-transform mass spectrometry (FTMS). The two mass analyzers were compared with regard to enantiomeric analysis. (Int J Mass Spectrom 219 (2002) 79–87)
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1. Introduction

Enantioselectivity in chemical reactions is a phenomenon based on interactions of groups around the chiral center. It is used as the basis for chiral separation and analysis of enantiomeric mixtures. The forefront of enantioselective separation has mainly been in the field of liquid chromatography specifically high performance liquid chromatography (HPLC). Selectors such as cyclodextrins are used in conjunction with HPLC to separate and quantify enantiomeric mixtures [1–4]. Similar systems have been extended to capillary electrophoresis (CE) [1–9] where the small column dimensions and the fast time scale of the separation makes it an extremely attractive method for chiral separation.

A purely mass spectrometry based method for the determination of enantiomeric excess, without the pre-chromatographic separation, would be advantageous, as it would be faster than chemical separations. Detection limits can be lowered without the need for derivatization. Information obtained would include structural and chemical information alongside the chiral analysis. Unlike in chromatography, where the selector is immobilized in large amounts in a column, in mass spectrometry the selector or host may be added into the analyte solution in approximately the same relative amounts. A much greater array of substrate choices results. Also, switching to new selectors can be done easily, without expensive column replacement. In mass spectrometry, analysis of nearly every type of compound is possible due to the vast array of ionization sources available.

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There have been numerous studies where mass spectrometry was used for the determination of enantiomeric excess by employing it as a detection tool for chromatography. For example, mass spectrometry with electron-impact ionization has been coupled with gas chromatography to determine enantiomeric excess [4,10–18]. Similarly, mass spectrometry with electrospray ionization has been coupled with HPLC [10,14,15].

Numerous approaches for the determination of enantiomeric excess (ee) have been put forth with mass spectrometry. Often the method consists of a host molecule complexing with an analyte guest bound by various non-covalent interactions such as H-bonding or van der Waals [19–23]. The resulting complexes are diastereomeric with unique ionization efficiencies [24,25]. The differences in ionization efficiencies in turn produce unique abundances that can be normalized to provide ee. Determination of ee may also be performed by collision-induced dissociation, where the MS/MS of diastereomeric adducts is performed [26,27].

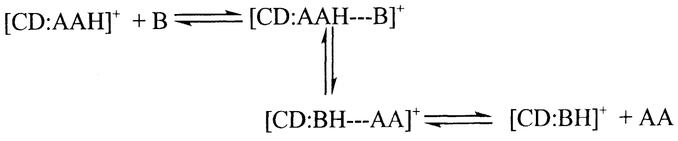
An enantioselective mass spectrometric method developed in our laboratory consists of a gas-phase guest-exchange reaction involving complexes composed of cyclodextrins and protonated amino acids reacting with neutral alkyl amines [28–32]. Cyclodextrins are cyclic carbohydrates that act as hosts for analyte guests (e.g., amino acids). In the gas-phase, in the presence of a gaseous alkyl amine, the amino acid (AA) was displaced by the alkyl amine (B) in a guest-exchange reaction to produce a new cyclodextrin:amine complex $[\mathrm{CD{:}B} + \mathrm{H}]^+$ (Scheme 1). Enantioselectivity was obtained because the rate constants vary depending on the chirality (L or D) of the amino acid. The resulting method is simple, fast and relatively accurate. The instrument employed for the chiral analyses was a Fourier-transform mass spectrometry (FTMS) instrument.

The next step in this research is to assess the applicability of our method to another type of mass spectrometer, the quadrupole ion trap. Quadrupole ion traps have gained wide acceptance due to their versatility and ease-of-use. Relative to FTMS, ion trap mass spectrometry (ITMS) instruments are more widely available. They have been used to determine enantiomeric excess by Leary and co-workers [25] and Cooks and co-workers [27,33]. In those methods, metal complexes consisting of analytes and selectors were collisionally dissociated and the relative abundances of specific products were related to the relative amounts of L- and D-enantiomers in the original mixture. In the current study, we describe the use of ITMS to monitor gas-phase enantioselective reactions. Although several groups have shown the versatility of ITMS for ion–molecule reactions [34–37], to our best knowledge there has been no previous report of enantioselective reactions with ITMS.

2. Experimental

For the chiral exchange reaction, the host used was permethylated cyclodextrin (heptakis-(2,3,6-tri-*O*-methyl)-β-cyclodextrin), purchased from Sigma Chemical Co (St. Louis, MO). Analyte guests were the following amino acids: Ala, Val, Leu, Ile, Phe, and Cys purchased from Sigma Chemical Co (St. Louis, MO), and used without further purification. The alkyl amine (B) used was *n*-propylamine (NPA), purchased from Aldrich Chemical Co. (Milwaukee, WI).

All experiments were performed on a modified Finnigan LCQ ion-trap mass spectrometer operating at a He background pressure of 1.75 mTorr [38]. The



Scheme 1. Proposed mechanism for gas-phase guest-exchange reactions.

CD (host) solution was prepared by dissolving the methylated cyclodextrin in a water/methanol, 50:50 solution, obtaining a concentration of 1×10^{-6} M. Analyte (guest) solutions, 1×10^{-6} M, were made by dissolving the amino acid in water/methanol. The $[CD{:}AA + H]^+$ electrospray solution was made by mixing a 10:1 ratio of analyte to host and was pumped at a rate of 2–5 μL/min. The electrospray needle was held at 4 kV and the heated capillary was kept at a temperature of 200 °C.

The NPA was introduced into the helium background gas using a manifold external to the ion trap that has been described previously [38]. A brief overview is given here. With a syringe pump, the liquid amine (35–150 μL/h) was introduced into a fast flow of helium (500–1500 mL/min). Rapid evaporation and mixing in the manifold lead to well-defined He/amine gas mixtures. When the gas mixture was applied to the LCQs restriction capillary inlet at 155 Torr, a pressure of 1.75 mTorr was obtained in the trap. To obtain lower effective flow rates, the amine was diluted in cyclohexane before being added by the syringe pump. The cyclohexane was inert and did not participate in the exchange reaction. The system allows for the easy preparation of mixtures with He/amine ratios from 100 to 10000:1.

The majority of the gas mixture was vented to an exhaust system while less than 1 mL/min flowed into the trap through the restriction capillary. The partial pressure of the NPA was calculated by dividing the He pressure (approximately 1.75 mTorr) by the mixing ratio and accounting for differential effusion. In this way, the overall reagent pressure was determined with an overall uncertainty of ±15%. Identical conditions were employed for the enantiomeric pairs.

The mass spectra were acquired in the following manner. The alkyl amine flow was set and the pressure was allowed to reach a steady state (several minutes). A mass spectrum was taken and the following ions were observed: reactant $[CD{:}AA + H]^+$ (base peak), $[CD{:}Na]^+$(70%), and $[CD{:}K]^+$ (50%), and product $[CD{:}B + H]^+$ (<5%). The software was then set to do tandem mass spectrometry (MS/MS) scans. This consisted of isolating the ion of interest $[CD{:}AA + H]^+$ using a notched waveform to eliminate all other ions. This reactant ion was then held in the trap for a specified reaction time period before ejecting all the ions to obtain a product mass spectrum. For each mass spectrum, 5–7 scans were averaged. To obtain kinetic data, a series of spectra (at least 10) were taken with varying reaction times and the intensities of specific *m*/*z* values were recorded. The mass spectra reflect the consumption of the $[CD{:}AA{+}H]^+$ as the $[CD{:}B{+}H]^+$ was produced. Reaction times were evenly spaced and chosen such that at the longest time, the reactant peak was below the noise level; however, the reaction was considered complete when the reactant peak was 5% of the product peak.

Table 1
Selectivities ($S = k_L/k_D$) of guest-exchange reactions with FTMS and ITMS

AA	FTMS	ITMS
Ala	1.6 ± 0.1	1.1 ± 0.1
Val	3.1 ± 0.1	1.9 ± 0.1
Phe	0.82 ± 0.1	1.0 ± 0.2
Leu	3.6 ± 0.1	2.9 ± 0.1
Ile	3.8 ± 0.1	2.9 ± 0.5
Cys	2.2 ± 0.1	3.0 ± 0.1

The exchange reaction rate constants *k*, shown in Table 1, were the result of averaging *k* values from at least four different runs. Measurements for each reaction were made at several alkyl amine pressures. To calculate *k*, $\ln\{[CD : AA + H]^+/([CD : AA + H]^+ + [CD : B + H]^+)\}$ was plotted vs. the reaction time, and the slope obtained from a least-squares fit. The second-order rate constant—units of cm^3/s molecule—was obtained by dividing the graph's slope by the number density of the NPA.

3. Results and discussion

3.1. Determination of rate constants with ITMS

The systems chosen were first analyzed using a FTMS instrument [29–31]. Reaction rate constants were determined when the complex $[CD{:}AA + H]^+$ was introduced into the ion trap and allowed to react with the alkyl base present. The product resulting from the initial reaction of the amino acid complex with the base present was observed immediately. Zero reaction time was set immediately after ejection of this product. After isolation, the intensities of the reactant and product mass spectrometric peaks were recorded as the reaction progressed. We collected data until the reaction was completed with the reactant completely disappeared. Throughout the reaction, non-reacting alkali metal complexes of sodium $[CD + Na]^+$ and $[CD + K]^+$ potassium were present and used as references to ensure no other reaction channels were present. Their peak intensities were constant, as was observed with the FTMS.

Fig. 1 shows a series of spectra for the reaction of $[CD:Ile + H]^+$ with NPA. The reactions for both enantiomers were complete in less than 10 s. At 1.5 s, the L -isomer complex (*m*/*z* 1560) was approximately 30% reacted to form the product $[CD:NPA + H]^+$ (*m*/*z* 1488) (upper spectrum, Fig. 1a). At 2.3 s, the reactant and the product had the same intensities, indicating 50% completion (middle spectrum, Fig. 1a). At 3.1 s, the product ion was the dominant peak (lower spectrum, Fig. 1a). The D -isomer reacted somewhat slower; the reaction was 50% complete at 3.9 s (middle spectrum, Fig. 1b). Both reactions were significantly faster than the same reaction performed in the FTMS, which required tens of seconds to complete at lower pressures of NPA.

The kinetic plots for both enantiomers of Ile in a reaction with NPA are shown in Fig. 2. These plots are representative of all the amino acid examined in this study. There are two important features that distinguish them from those obtained from FTMS. First, the reaction times are significantly shorter for the ITMS. To obtain rate constants for the reaction of the L -enantiomer of isoleucine with NPA, for example, took 5 s on the LCQ compared to 160 s for the FTMS. The pressure in the FTMS can be increased to speed up the reaction. However, there are limitations as significantly high pressure ($>10^{-6}$ Torr) degrades FTMS performance. The quadrupole ion trap mass analyzer has less stringent pressure requirements allowing reactions to be performed at significantly higher pressures. Second, the coefficient of correlation (r^2) is typically less in the ITMS experiment (0.986 and 0.987 for both enantiomers) compared to FTMS (0.999).

3.2. Enantioselectivity determined by ITMS

Fig. 3 shows the rate plots for the $[CD:Phe + H]^+$ complex reacting with the base, NPA, to produce the complex $[CD:NPA + H]^+$. The ratio of the rate constant (k_L/k_D) was near unity (1.0) and close to that obtained by FTMS (0.82). The enantioselectivity values ($S = k_L/k_D$) listed in Table 1 were averages of at least four determinations. The low selectivity for Phe has been attributed to the relative size of the phenyl side chain and the β-cyclodextrin cavity [28,29]. The relatively rigid cavity of β-cyclodextrin constrains the phenyl group, restricting the interactions of ammonium and carboxylic acid functional groups with either upper or lower rim of cyclodextrin. Molecular modeling calculations supported this notion and predicted that the interactions of both enantiomers with the β-cyclodextrin host would be very similar possibly resulting in low enantioselectivity [28,29].

For the other amino acids, the same trends were observed between selectivities in both ITMS and FTMS. For Ala, the selectivity was lower with the ITMS than with the FTMS (1.1 and 1.6, respectively). The selectivity for the ITMS increased for Val and Cys (1.9 and 3.0, respectively) as it did for the FTMS (3.1 and 2.2, respectively). Interestingly, for Cys the selectivity was higher for ITMS than FTMS, while for all other amino acids the FTMS values were larger. For Leu and Ile the values increased further with ITMS (2.9 for both), as it did with FTMS (3.6 and 3.8, respectively). Despite the generally lower selectivity obtained with the ITMS, the trend for both analyzers were maintained. That is, as the size of the side chain increased, the selectivity increased with the exception of Phe. However, because of the finite cavity size associated with β-cyclodextrin, an optimal guest size was reached with Leu and Ile to produce the maximum selectivity. Increasing the size of the guest further, as with Phe, decreased the selectivity considerably [28,29].

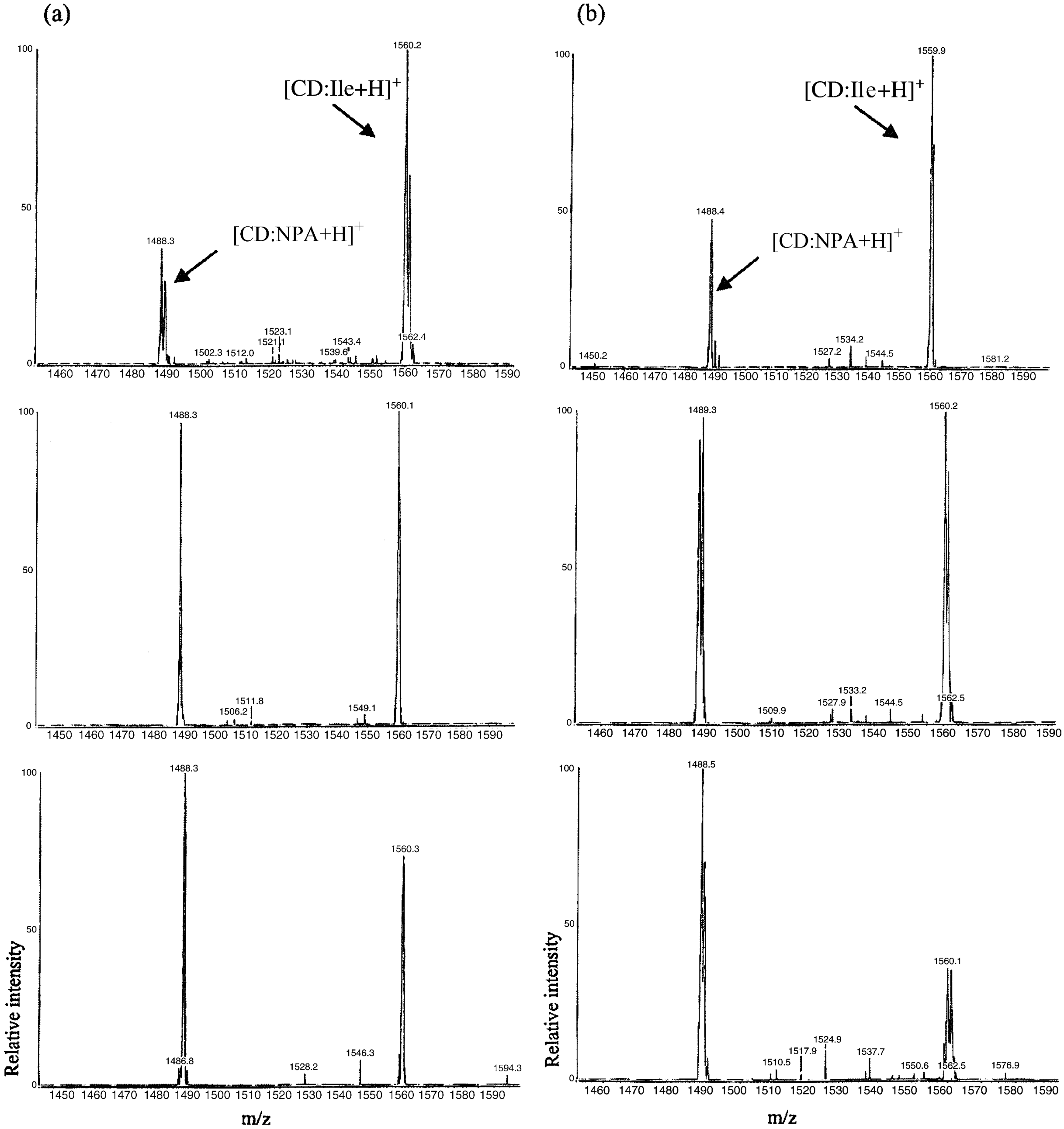


Fig. 1. Representative spectra obtained on an LCQ instrument ion-trap mass spectyrometer of [CD:Ile + H]$^+$ reacting with NPA reagent gas. (a) The spectra are from the L -isomer with reaction times of 1.5 s (upper), 2.3 s (middle), and 3.1 s (lower). (b) The spectra are from the D -isomer with reaction times of 3.1 s (upper), 3.9 s (middle), and 6.8 s (lower). The L -isomer was more reactive than the D . Both reactions were complete in less than 10 s.

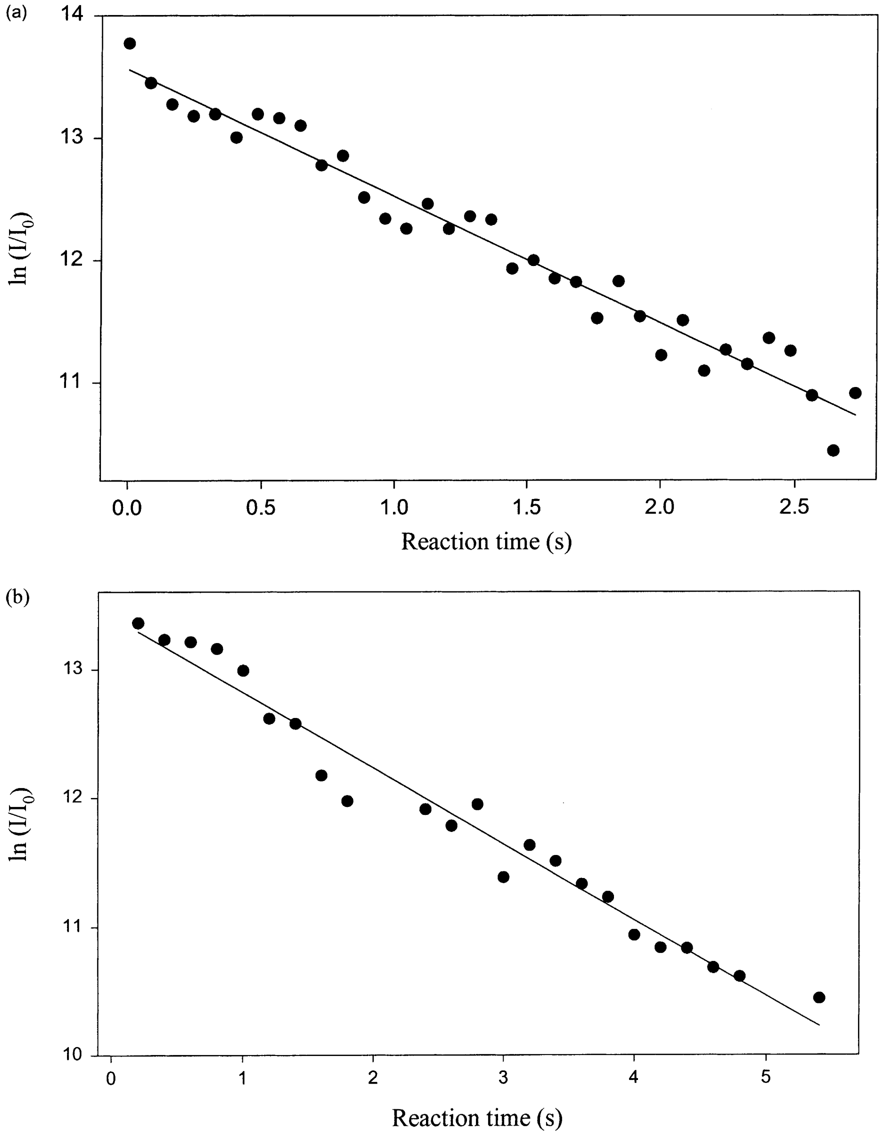


Fig. 2. Kinetic plots of [CD:Ile + H]$^+$ reacting with NPA for the L -isomer (a) and the D -isomer (b). The r^2 values are 0.963 and 0.970, respectively.

4. Discussion

Enantioselectivity with ITMS was obtained in the gas-phase guest-exchange reaction outlined in Scheme 1. The trend in the selectivities was consistent with the relationship between the size of the side chain and the size of the cyclodextrin cavity, i.e., the selectivity increased from Ala to Val, Leu and Ile. Phe was a special case as it has a sufficiently large phenyl side chain that was severely constrained by the dimension of the β-cyclodextrin cavity.

Selectivity values, *S*, obtained with ITMS were generally smaller than those obtained with FTMS for amino acids with large alkyl side chains. For example,

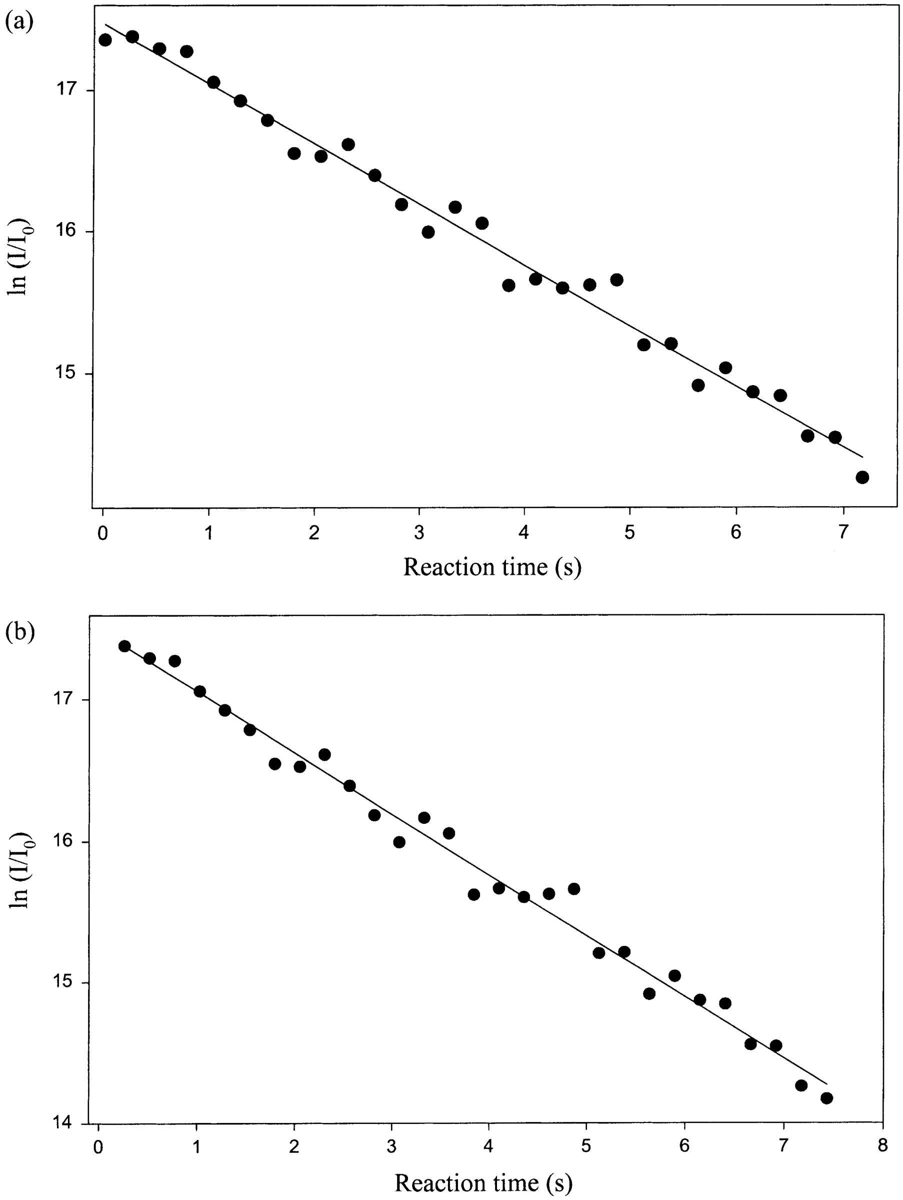


Fig. 3. Kinetic plots of $[\mathrm{CD{:}Phe+H}]^+$ reacting with NPA for the L -isomer (a) and the D -isomer (b). The r^2 values are 0.986 for both plots.

Leu with the FTMS yielded a value $S = 3.6$ whereas with the ITMS the corresponding value was 2.8. Similarly for Ile the respective values were 3.8 and 3.2. The source of the lower selectivities was not readily apparent but may be due to the distinct differences between the two types of mass analyzers. Mass analyses in the FTMS were performed at 10^{-7} Torr while those in the ITMS were performed at 10^{-3} Torr. The higher pressures increase the rate of three-body collisions, which may affect the relative reaction rates.

Differences in the reaction temperature may also account for the lower selectivities. The temperature of the ITMS may be slightly higher than that of the FTMS making the ions in the former more energetic. Several ionic species with variations in conformations and interactions between the amino acid guest and the cyclodextrin host may be present with slight structural variations. The higher energy structures may produce more reactive species that result in little or no enantioselectivity. The FTMS experiments were performed at room temperature or 298 K. Recent analysis of the reaction temperature in the ITMS indicated that it may be 310 ± 20 K [39], which, although quite low, may still be potentially higher than the ambient temperature of the FTMS.

5. Conclusion

Enantioselectivity was obtained in the gas-phase guest-exchange reaction utilizing ITMS. The ion trap mass spectrometer has proven to be amenable for the investigation of enantioselective gas-phase ion–molecule reactions. The enantioselective guest-exchange reaction employing a cyclodextrin host and an amino acid guest is a general phenomenon and is not limited to FTMS. Although the selectivity in ITMS is less than in FTMS, it is sufficiently large and should be suitable for creating a calibration table. The method itself could be general and applied to a number of chiral analytes. ITMS also has an inherent advantage. The relatively high reactant gas pressure achieved with ITMS allows for faster reactions. Analysis could therefore be completed more rapidly. The shorter duration of the reaction could be advantageous particularly in high throughput analyses.

These preliminary results warrant a more systematic study involving other amino acids and other oligosaccharide hosts. A further fine-tuning of the reaction parameters in the ion trap may further increase enantioselectivity.

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