International Journal of Mass Spectrometry 428 (2018) 1–7

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

journal homepage: www.elsevier.com/locate/ijms

Full Length Article

Chiral recognition of amino acid enantiomers using high-definition differential ion mobility mass spectrometry

J. Diana Zhang, K.M. Mohibul Kabir, Hyun Eui Lee, William A. Donald *

School of Chemistry, University of New South Wales, Sydney, NSW, 2052, Australia

ARTICLE INFO

Article history:
Received 15 August 2017
Received in revised form
25 December 2017
Accepted 7 February 2018
Available online 13 February 2018

Keywords:
Chiral recognition
Enantiomer separation
Differential ion mobility
High-field asymmetric waveform ion mobility
Ion mobility spectrometry
Mass spectrometry

ABSTRACT

Enantiomeric analysis of small molecules is important in many research fields, including in drug development. Here, chiral recognition of amino acid enantiomers using differential ion mobility spectrometry (DMS) mass spectrometry (MS) is demonstrated. Diastereomeric proton bound complexes were formed between enantiomers of amino acids (tryptophan and phenylalanine) and *N*-*tert*-butoxycarbonyl-*O*-benzyl-L-serine (BBS) by electrospray ionization for analysis by DMS-MS and collision-induced dissociation (CID). If the DMS resolution is sufficiently high, ionic diastereomers (as opposed to enantiomers) can in principle be separated without the use of a chiral gas. Peaks corresponding to the *L*- and *D*-enantiomers for both tryptophan and phenylalanine in the DMS-MS spectra were resolved by increasing the ratio of He in the carrier gas from 100% N_2 to 50:50 He:N_2%. In contrast, CID spectra of the corresponding diastereomeric dimer complex ions were nearly identical, indicating that chiral recognition by CID was not possible under these conditions. For mixtures of *L*- and *D*-tryptophan, a linear calibration curve can be obtained by plotting the enantiomeric excess measured by DMS-MS *vs.* the known values in solution (slope of 1.000, intercept of −0.010 and R^2 of 0.997). That is, enantiopurity can be quantified using a separation process that occurs in milliseconds. Thus, DMS-MS analysis of proton bound diastereomeric dimers is a powerful approach for the rapid enantiomeric analysis of relatively small molecules.

1. Introduction

Chirality is naturally embedded within many biomolecules, including amino acids, sugars and proteins, and is essential to proper cellular and biological function [1,2]. Molecular chirality is of fundamental importance in drug discovery [1,3]. For example, more than 50% of drugs that are brought to market have chiral centers, and of these, *ca.* 50% are administered as racemates [4,5]. However, pure enantiomeric forms of drugs are often needed in order to produce a desired therapeutic effect because the biological activity of enantiomers can differ significantly in a chiral environment, including many ligand-protein binding motifs [6,7]. For example, *R*-thalidomide is an effective anti-nausea drug, whereas *S*-thalidomide can cause birth defects [4]. Thus, newly developed drugs, as recommended by the Food and Drug Administration (FDA), are required to undergo intensive enantiomeric analysis [8,9]. FDA guidelines emphasize the need for rapid, robust and sensitive methodologies in both qualitative and quantitative analyses during drug development [1,10]

In order to achieve enantiomeric separation of chiral molecules, enantiomers need to be subjected to an asymmetric chiral environment; *i.e.*, an environment that promotes stereoselective interactions. Enantioselectivity requires at least three-points of intermolecular interactions between an enantiomer and a chiral environment such that at least one interaction is stereochemically dependent, which is known as Pirkle's rule [11]. One application of Pirkle's rule is to form a diastereomeric complex by complexing a chiral selector molecule with an enantiomer. Thus, enantioselectivity can in principle be achieved by using analytical instrumental methodologies that are sensitive to the different thermodynamic energies and structures present in the binding of the 'analyte' enantiomer to the chiral selector [11,12].

Several mass spectrometry (MS) methods for chiral recognition have been developed for rapid, selective and sensitive detection of chiral analytes from chemical mixtures [1,2,10]. These MS chiral recognition methodologies can be divided into three main categories: (i) determining the relative abundance of diastereomeric adduct ions that are formed between an enantiopure reference compound and analyte enantiomers; (ii) using collision-induced

* Corresponding author.
E-mail address: w.donald@unsw.edu.au (W.A. Donald).

https://doi.org/10.1016/j.ijms.2018.02.003
1387-3806/

dissociation (CID) to identify any differences in the kinetic stability of diastereomeric complex ions; and (iii) using gas-phase ion molecule reactions to identify differences in thermodynamic or kinetic constants of reactions that depend on chirality [2,10,12,13]. For example, Yao et al. [14] measured the difference in the relative product ion abundances in the CID mass spectra of size-selected diastereomeric proton bound trimer complex ions for the chiral recognition of amino acids. However, such methods require the formation of relatively large and weakly bound complex ions for optimal chiral recognition. Moreover, the CID fragmentation patterns of many diastereomeric complex ions that differ by the presence of an *L*- and *D*-enantiomer are indistinguishable [14,15].

Ion mobility spectrometry (IMS) is an emerging technique for gas-phase chiral analysis [1]. In IMS, gas-phase ions can be separated and detected based on their mobility as they pass through a buffer gas under the influence of a constant weak electric field [12,16]. The mobility of an ion in a given carrier gas can depend on the mass (m), charge (z), and collision cross-section (CCS) of the ion, the carrier gas, and the electric field strength [2,13,17–19]. In conventional IMS, an electric field lower than 100 V/cm is used to ensure that ion mobility is independent of the electric field strength [20]. Thus, ions can be separated because the drift velocity of the ions depend on their m, z, and CCS. IMS based ion separations can be several orders of magnitude shorter than common methodologies for chiral separations, such as high-performance liquid chromatography [13,21]. Furthermore, as an orthogonal approach to MS, IMS can be used to separate ions prior to mass analysis, which can increase ion signal-to-noise and lower limits of detection [22,23].

In the last decade, there has been a strong interest in developing IMS as a robust method for enantiomeric analysis [1,22]. Recently, research in this field has primarily involved the analysis of relatively large biomolecules such as epimeric glycans [24], catechin epimers [17], carbohydrate anomers [16], and mono- and disaccharide isomers [25]. Studies regarding the enantiomeric analysis of small molecules such as amino acids and those relevant to drug development have been limited. To date, there has been four reports of IMS being used for chiral separations in the literature [2], two of which involves relatively well-established IMS based instruments; *i.e.*, drift tube ion mobility-mass spectrometry (DTIMS) [3] and travelling wave ion mobility-mass spectrometry (TWIMS) [13]. By use of IMS, enantiomeric amino acids have been separated by using (i) an enantiopure amino acid as a reference compound complexed with a divalent metal, to form metal bound trimers [13,18,23]; and (ii) a chiral modifier that was doped into the drift gas, which resulted in different ion mobilities for each enantiomer ion upon ion-molecule clustering with the chiral gas modifier [3]. However, these IMS methods can be limited by (i) the formation of weakly bound and relatively large complex ions (*i.e.*, trimers), which require the optimization of concentration ratios between a reference compound, metal ion, and chiral selector; (ii) relatively high analyte concentrations; (iii) the use of relatively 'soft' ionization methods that favor ion formation of polar molecules (*e.g.*, electrospray ionization, ESI); and (iv) insufficient resolution of the *L*- and the *D*- enantiomers for direct and accurate quantitation [26]. In addition, the prolonged use of a suitable chiral buffer gas dopant can be relatively expensive compared to the use of solid chiral selector molecules, such as modified amino acids with a *tert*-butoxycarbonyl (Boc) protecting group (*e.g.*, *N*-*tert*-butoxycarbonyl-*O*-benzyl-L-serine; BBS, where Bzl is a benzyl group) [14]. Thus, the exploration of alternative MS based methods for rapid chiral analysis is warranted.

One type of IMS based methodology that has undergone considerable improvement in resolving power is differential ion mobility spectrometry (DMS), otherwise known as high-field asymmetric waveform ion mobility spectrometry or FAIMS (Fig. 1). DMS utilizes an alternating high and low electric field to separate gas-phase ions, in which the high-field portion of the electric field is greater

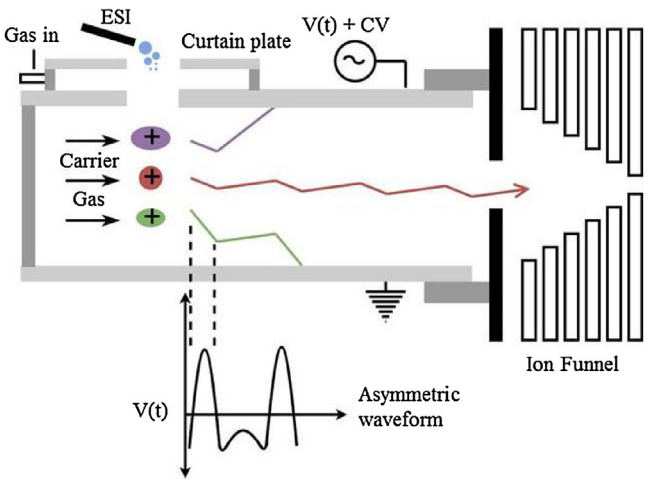

Fig. 1. Diagram of differential ion mobility and the ion funnel interface to a linear quadrupole ion trap mass spectrometer.

than 10 kV/cm [20]. Under the influence of a high electric field, the mobility of an ion depends nonlinearly on the electric field [27]. In DMS, a high frequency alternating asymmetric voltage is applied between two electrode plates and a longitudinal gas flow 'carries' ions between the two plates (Fig. 1) [18]. For the asymmetric voltage, the high-field is applied for a short duration and a lower voltage of opposite polarity is applied for a longer duration. The peak amplitude of the high-field portion is referred to as the dispersion voltage (DV). In the resultant 'dispersion field' ($E_D = DV/d$ where d is the distance between the DMS electrodes), ions migrate towards one electrode under the high-field portion of the waveform, and reverse direction towards the other electrode during the low-field portion of the waveform. By superimposing a constant and adjustable DC compensation voltage (CV), resulting in a 'compensation field' (E_C), onto the asymmetric waveform, the net displacement of the ion caused by the different ion mobilities in the high and low electric fields can result in the transmission of different ions between the plates. By scanning a range of compensation voltages, a spectrum of the relative number of ions that have traversed the gap can be obtained as a function of CV [18,22,28–31].

Some advantages of DMS include: (i) ion separation is more orthogonal to MS than other IMS based methods because ion separation depends less strongly on m and z, and more on the electric field [32]; and (ii) the peak capacity of DMS can be relatively high compared to other IMS based techniques [22]. In DMS, the resolving power for a given peak is defined as the ratio of E_C and the full-width-at-half maximum of the peak. The resolving power of DMS-MS was initially limited to ~10 [28], partly due to the use of curved (cylindrical or spherical) electrodes that resulted in inhomogeneous electric fields between the plates [31]. However, the resolving power values for multiply charged peptide ions as high as ~500 has been reported by the use of 'high-definition' DMS-MS [31]. The improvement in resolving power in high-definition DMS-MS has been attributed in part to the use of: (i) planar electrodes for more homogeneous electric fields than for curved electrodes (cylindrical FAIMS) [28]; (ii) relatively high concentrations of He and H_2 in the carrier gas, which results in higher ion mobilities than by use of heavier carrier gases (and non-Blanc ion mobility behavior) [28,30,33,34]; and (iii) higher E_D values than were used previously, which enhances ion separation [31,35].

The use of DMS-MS for chiral recognition has been reported twice by Reimann and co-workers [18,23], which corresponds to the third and fourth reported example of using any IMS based method for chiral analysis. In these studies [18,23], cylindrical FAIMS was used to partially separate metal bound trimer com-