A Comparison of Sequential Fragmentation at High Energies with True MSn Analysis Utilizing Energy Dependent Breakdown Curves

Poster Note 64496

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Overview

Purpose: To study the ability of a breakdown curve of incremental energy applied during high energy collisional dissociation as an indicator of true sequential fragmentation.

Methods: HRAM fragmentation data was acquired on several hundred compounds which included both high energy collisional dissociation (HCD) and trap collision induced dissociation (CID). HCD MS^2 data was acquired at incremented energies from 10 to 200% normalized to the mass of the precursor and CID MS^2 and MS^n data was acquired in a modeled fashion for the precursor.

Results: For molecules where fragmentation proceeded primarily from subsequent concurrent fragmentation, the breakdown curves created by incrementing the HCD energy could provide significant insight to fragmentation relations. However, this was not often observed to be the case as often competing concurrent fragment pathways typically created multiple energy dependent formation relations which complicated direct interpretation of limited MS2-only data.

Introduction

Many fragmentation mechanisms are possible in modern mass spectrometers but the two most common mechanisms rely on the transfer of energy to the molecule by collision with a neutral gas. These mechanisms differ significantly in the mechanism by which the energy is deposited however which leads to differences in the style of fragments generated. The two approaches studied were high energy collisional dissociation (HCD) which is typical of triple quadrupole instruments or any instrument using a quadrupole collision cell as well as instruments using an HCD cell, and collision induced dissociation (CID) which is common for both 2D and 3D ion traps. Our goal was to study the concurrent fragmentation that can happen during HCD (generation of either competing fragments of subsequent fragments) vs sequential fragmentation that occurs with the isolation and reactivation of a fragment ion (typically referred to as "MS^n" fragmentation). To do this, we made use of incremental energy breakdown curves for HCD fragmentation and compared the energy dependency of concurrent subsequent fragments with true sequential MS^n

Methods

Data Acquisition

All fragmentation data was acquired on pure standards of compounds infused into an Orbitrap mass spectrometer (Thermo Scientific™ Q Exactive™ MS and Thermo Scientific™ Orbitrap Elite™ MS). Purpose built software tools were used to control the instrument in real time to acquire replicate scans at each collision condition and to increment the collision energy automatically (QETool and TreeRobot).

HCD Breakdown Curves

For high energy collisional dissociation (HCD), energy was applied at twenty different incremented levels normalized to the mass to charge of the precursor.

CID Fragmentation Energy

Trap collision induced dissociation (CID) fragmentation data as acquired at incremented energies for the MS^2 level and at an optimized energy for subsequent MS^n levels due to the nature of CID fragmentation.

Data Processing

At least three scans were taken at each energy level and the replicate scans were used to determine noise in the signal and filtered averages were created. Finally, the *in silico* predicted fragments and elemental compositions for the observed fragment ions were used to create a recalibration for every spectra individually and applied to the filtered data to create recalibrated spectra. Breakdown curves for HCD fragmentation were created by plotting the intensity, both absolute and relative, for each fragment ion against the energy applied. All data was hosted and investigated on mzCloud™ (www.mzCloud.org).

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Results

Clarification of Nomenclature

Sequential vs. Concurrent Fragmentation

For the purposes of this work, sequential fragmentation is used to mean fragmentation events that happen with isolation of an ion prior to creation of the next generation of fragments from it. This can be considered to be "true MS^n". Further, concurrent fragmentation here refers to fragmentation events that occur within a single measured scan event. These may arise from two different situations, competitively or subsequently.

Competing vs. Subsequent Mechanisms and Pathways

Competing concurrent fragmentation is taken to mean fragmentation which arises from different pathways with separate kinetics while subsequent concurrent fragmentation is taken to mean fragments that arise from the same pathway.

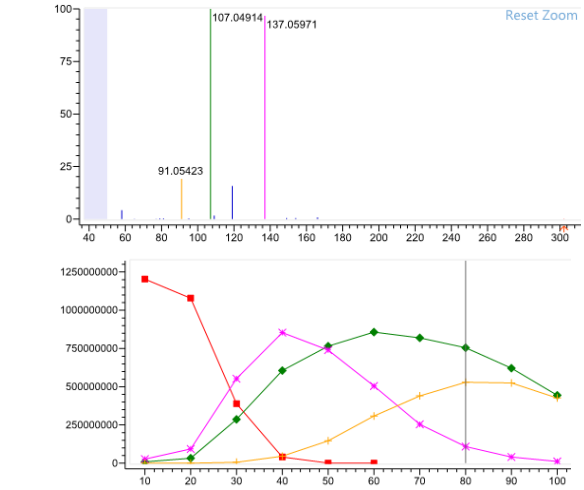
Mechanisms of Fragmentation

It is important to consider the basic differences between the fragmentation mechanisms used in this study – high energy collisional dissociation (HCD) and trap collision induced dissociation (CID). The nomenclature is not common across various different instruments so an initial clarification is undertaken here. In addition, for this work a single precursor ion was isolated for fragmentation, we are not considering wider isolation / non-precursor selective fragmentation mechanisms in this work.

For the purposes of this work, HCD is the fragmentation technique in which incoming precursor ions are subjected to a voltage difference from the ion optics to a collision cell. The greater the difference in voltage, the higher the acceleration the incoming precursor ions are subjected to. This increase in velocity makes the collisions with the "static" gas in the collision cell more energetic at higher voltage differences and imparts more energy to the precursor ion. Depending on the energy setting, precursor ions can be imparted with significantly higher energies and first generation fragments created may still retain enough internal energy to undergo subsequent fragmentation event(s) creating subsequent concurrent fragments.

Trap collision induced dissociation (CID) proceeds through the trapping, cooling, and subsequent resonance excitation of a specific precursor m/z value. As it collides with gas molecules, the compounds internal energy rises until it has enough energy to access the fragmentation pathways with the lowest kinetic barrier. As a result, CID fragmentation tends to make fewer fragments and those created are generally from the energetically most favorable pathways.

FIGURE 1. Dobutamine HCD MS2 Breakdown Curve



Top: The fragment spectra for Dobutamine with the three primary fragment ion m/z values
Bottom: Breakdown curve showing the dependency on higher energy to create subsequent fragments at lower m/z values.

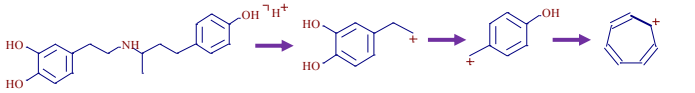
Breakdown Curve and Subsequent Concurrent Fragmentation: Dobutamine

As an introduction to the breakdown curve and an example of subsequent concurrent fragmentation, dobutamine was used as an example. The HCD MS2 fragment spectra and the breakdown curve are shown in Figure 1. The three major fragment ions from dobutamine are subsequent concurrent fragments with the fragmentation of parent following the sequence:

302.1751 → 137.0597 → 107.0491 → 91.0842

The structures of the fragments are shown in figure 2. As can be seen by the breakdown curve in Figure 1 and the pathway in Figure 2, increasing the HCD collision energy leads to the creation of fragment ions with sufficient residual energy to create subsequent concurrent fragments, MSn fragments in a nominally "MS2" scan.

FIGURE 2. Fragment Pathway for Dobutamine Subsequent Concurrent Fragments.

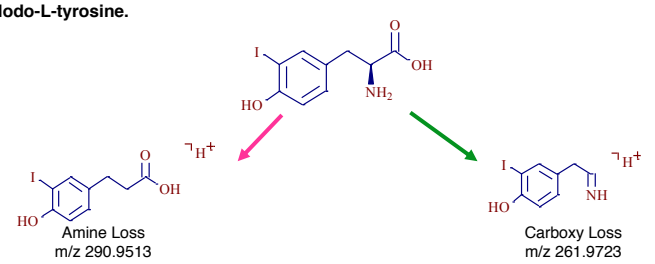


m/z: 302.1751 Max HCD: 10% | m/z: 137.0597 Max HCD: 40% | m/z: 107.0491 Max HCD: 60% | m/z: 91.0542 Max HCD: 80%

Indirectly Determining Kinetics for Competitive Concurrent Fragmentation

Fragmentation is a kinetic event where a precursor with sufficient energy will have one or more pathways available to it and the intensity of the resulting fragment ions is an indirect measure of the kinetic favorability of each pathway, assuming that no subsequent concurrent fragmentation occurs. Since such subsequent fragmentation is common in HCD, we have tried to use the relative maximum of breakdown curves as a means to determine which pathway is more favorable. As an example of this, we consider two pathways for 3-iodo-L-tyrosine, the first step of which is shown in Figure 3 with the HCD MS2 breakdown curve. The later maximum for the m/z 261.97233 fragment (loss of the carboxylic group) would indicate that this event requires a higher energy while the rapid maximum at only 10% for the loss of the amine and quick depletion would indicate this is energetically more favorable. As a second measure, we compare the CID formation for these fragments in MS2 (Figure 4) and indeed the formation of the amine loss (m/z 290.9513) is more prevalent.

FIGURE 3. Competing Concurrent Pathways and HCD MS2 Breakdown for 3-Iodo-L-tyrosine.



Amine Loss m/z 290.9513 | Carboxy Loss m/z 261.9723

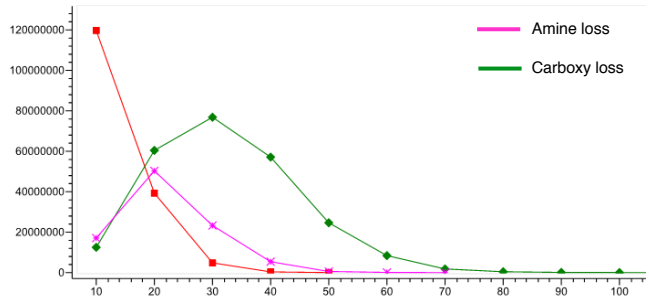
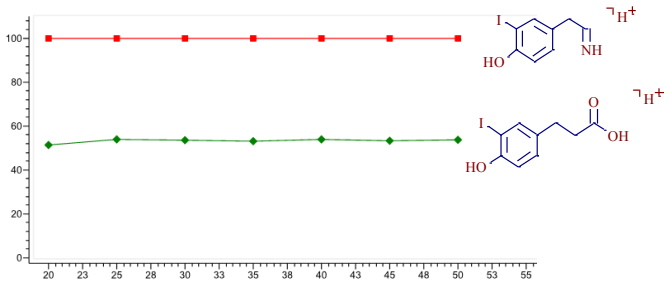


FIGURE 4. CID MS2 Breakdown Curve for 3-Iodo-L-tyrosine

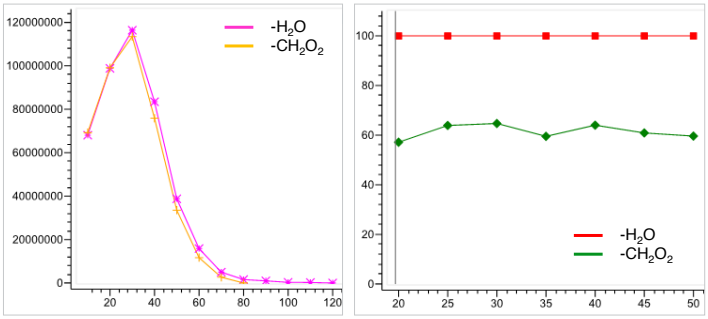


Note: Due to the mechanism of CID fragmentation, there is no subsequent energy dependence for fragmentation and the relative level is a surrogate for formation kinetics.

Overlapping and Concurrent Pathways

The data from 3-Iodo-L-tyrosine showed a competitive first fragment event where the products could not reasonably interconvert. Indole-3-lactic acid provides a similar example of two competing concurrent steps where one product can undergo subsequent concurrent fragmentation. Figure 5a shows the breakdown curves for the HCD MS2 data for these two fragments, the loss of water to form a terminal carbonyl (m/z 188) and the loss of the carboxylic function (m/z 160), which indicate that they have nearly the exact same formation dependence. The CID breakdown values in Figure 5b would indicate however that the formation of the water loss product (m/z 188) would be more favorable.

Figure 5. Breakdown Curves of Indole-3-Lactic acid



A: HCD Breakdown Curves B: CID Breakdown Curves

The apparent disconnect between the CID information that the formation of the water loss product (m/z 188) should be significantly favorable, and thus have a lower HCD maximum than the less favorable carboxylic acid loss (m/z 160), can be reconciled when we investigate the subsequent fragmentation for both these product ions. Figure 6 shows the observed fragmentation pathways determined by an investigation of the HRAM CID MSn fragmentation tree. Subsequent fragmentation of the water loss MS^2 fragment provides two major fragment ions, one from the loss of water (m/z 170) and the second from a rearrangement (m/z 146). It is interesting to note that, while observed in the CID MS^3, the formation of the carboxylic loss ion (from loss of the carbonyl) is NOT significant. This indicates that the pathway is more competitive than subsequent as indicated in Figure 6. The carboxylic loss product creates a single major fragment ion in MS^3 corresponding to the loss of the remainder of the lactic side chain. It should be noted that the CID MS^4 data from the water loss product also indicated this to be a major possible fragment which shows the collapse of the competing fragmentation pathways available at higher HCD energies.

If we investigate the HCD energy dependent breakdown curves for these ions (Figure 7.) we see that clearly the formation of m/z 118 is predominant. The water loss fragment has access to three subsequent fragment channels, each with a lower energy requirement, while the carboxylic acid fragment only has easy access to a single channel. This may account for the apparent overlap in formation curves observed in the HCD MS^2 data as the more readily formed water loss ion quickly undergoes subsequent concurrent fragmentation down multiple pathways.

Figure 6. Fragmentation Pathways for Indole-3-Lactic acid – Subsequent and Competitive

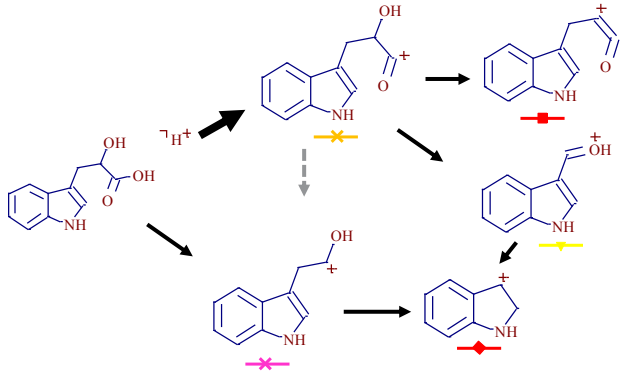
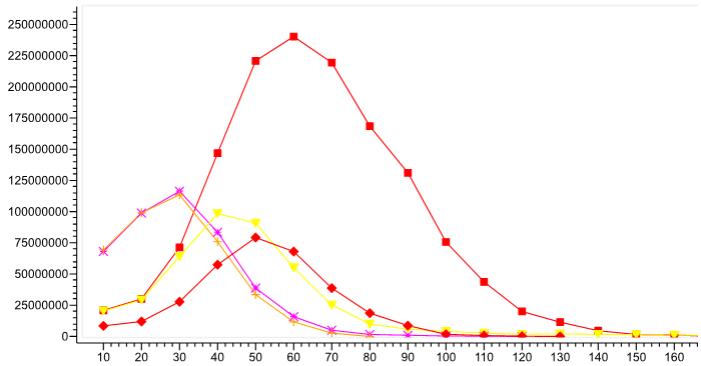


Figure 7. HCD MS^2 Breakdown Curves for the First and Second Step Fragments of Indole-3-Lactic acid



Conclusion

While HCD MS^2 fragmentation can provide a wealth of fragment ions resulting from the high energy deposition to the precursor opening multiple concurrent fragment pathways, the lack of true precursor to product ion relation can make interpretation difficult. Here we have investigated the use of energy-dependent breakdown curves as a means to elucidate the relationship between fragment ions that arise from subsequent or competitive concurrent events.

- In some simple cases, the breakdown curve based on HCD fragmentation can be sufficient to determine the subsequent concurrent fragment ions from a single precursor.
- The frequent presence of both subsequent and competitive concurrent fragmentation in HCD MS^2 complicates the ability to definitive parent-product relationship without subsequent fragmentation information.
- HCD-based MS^2 can provide useful information, especially for library identification, however it
- Subsequent MSn fragmentation derived from instrument-driven product ion selection and reactivation provides unambiguous