Utilizing UVPD Fragmentation for Plant Molecules: Phenylpropanoids

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ABSTRACT

Purpose: Investigate the potential use of UVPD to provide unique and potentially diagnostic fragmentation information for structure determination of small molecules, specifically phenylpropanoids and chalconoids.

Methods: Fragmentation data was acquired on several pure standards of compounds and for a matrix sample submitted to fragmentation by high energy collisional dissociation (HCD) and ultraviolet photodissociation (UVPD). All data acquired was high resolution accurate mass data and comparison of fragmentation (HCD) was made against a reference standard library.

Results: Laser-induced fragmentation provided unique fragments or enhanced the detection of kinetically unfavorable fragment ions in many of the compounds analyzed. These unique fragments provide additional information on the compounds studied which could be used to infer structure information.

INTRODUCTION

The determination of structure for unknown small molecules is a particularly difficult challenge. Typically, fragmentation data is acquired and used to compare against a reference spectral library or provide information on potential matches. However, when the reference libraries do not contain the molecule in question, similarity searches can provide an indication of possible substructure or structure class. These approaches are limited when considering compounds like plant secondary metabolites where there is significant chemical space and structure diversity and limited reference library coverage. Acquisition of high resolution accurate mass fragmentation data combined with substructure/similarity searching can provide clues to previously unknowns. Furthermore, the ability to access more unique fragments increases structural information. Typical fragmentation techniques rely on collision induced dissociation but ultraviolet photodissociation, in which a laser is used to provide the energy to a trapped molecular ion to drive fragmentation, could provide additional structure information by accessing unique fragment pathways. Here we apply UVPD to several small molecules to determine its utility for structure determination of small molecules.

MATERIALS AND METHODS

Sample Preparation

Standard material for 5 chalconoids (chalcone, 4-methoxy chalcone, butein, *trans*-clovamide, and naringeninchalcone) and 5 phenylpropanoids (caffeic acid, methyl caffeate, chlorogenic acid, *trans*-2-hydroxycinnamic acid, and *trans*-cinnamic acid) were prepared by dissolving in 1:1:1 ACN:MeOH:water to a final concentration of 0.5 ug/mL.

Mass Spectrometer Acquisition Conditions

Mass spectrometer: Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid MS with UVPD

UHPLC: Thermo Scientific™ Dionex™ Ultimate™ U3000 HPG pump and WP autosampler

Laser: Class 1 213nM, 2.5kHz at 1.2μJ/pulse

Mobile Phase A: Water with 0.1% formic acid

Mobile Phase B: Acetonitrile with 0.1% formic acid

Flow rate: 450 uL/min

Table 1. LC Gradient

Time (min)	% A	% B
0.0	98	2
0.5	98	2
7.5	5	95
8.0	5	95
8.5	98	2
10.0	98	2

UVPD Laser

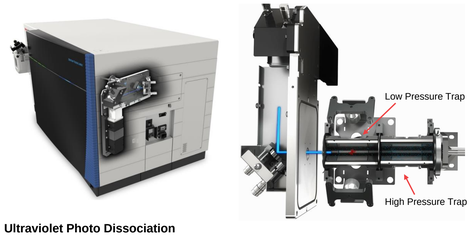
For this work, we used a Nd:YAG (neodymium doped yttrium aluminum garnet) laser. This is an optically pumped laser that typically emits in the infrared range (>1000nm). When operated in a pulsed Q-switching mode, where the laser energy is released in a pulse when reaching a threshold, frequency doubling of the pulses can be used to obtain shorter wavelengths. The laser in this study used the 5th harmonic of the Nd:YAG fundamental, resulting in a radiated wavelength at 213nm.

The laser was mounted such that the path entered the dual linear ion trap with fragmentation occurring in the high pressure region (Figures 1). The laser energy imparted could be adjusted by increasing the pulse count for each fragment scan. For this work, we applied 125, 375, and 750 pulses which equates to 50, 150, and 300 msec respectively. These may seem relatively long times when compared to resonance excitation fragmentation (trap CID) or to trapping-based collision induced fragmentation (HCD) style devices, however the applied energy of either collision based method is the result of multiple collisions (through resonance excitation or voltage offset acceleration) while UVPD, each photon carries 5.3 eV to the target molecule. The current laser, at 1.2 μJ/pulse, generates approximately $1.3 \cdot 10^{12}$ photons per pulse which is significantly higher than the total number of target molecules (in the range of $1 \cdot 10^{5}$ to $1 \cdot 10^{6}$) for even a single pulse. Of course, consideration must be given to the cross section of the ions in the trap and the laser pulse. The difference in the mechanism of imparting energy has an observable difference in the type and extent of fragmentation observed.

Figure 1. Orientation of the lasers onto the Orbitrap Fusion Lumos Mass Spectrometer

Exterior right side view of the mass spectrometer showing mounting of the laser.

Top-down view of the laser path (blue line) through the linear ion trap low and high pressure cells.



Ultraviolet Photo Dissociation

Photodissociation utilizes the energy of incident photons (in this case, 5.3 eV/photon) to increase the internal energy of a target molecule until it has sufficient energy to overcome its barrier for dissociation. As opposed to collisional induced fragmentation mechanisms where energy is imparted through random collisions of the molecular ions with gas molecules, the photons in UVPD are absorbed by target molecules depending on their chemical structure and the wavelength of the photon. Typically a single photon does not impart sufficient energy for fragmentation and the absorption of multiple photons is typically required to reach a sufficiently excited state. This is why UVPD is typically also referred to as infrared multiphoton dissociation (IRMPD). It is important to consider the specific energy required for a given structure class of molecules (the result of the number of photons and the energy per photon) may be higher than a single photon, but the incident laser pulses contain far more photons than target molecules by several orders of magnitude so multiple photons may strike a molecule per pulse. The wavelength of the laser used for this work, 213nm, falls near the range of several relevant chemical substructures (Table 2) for compounds of interest.

Table 2. Absorbance Values for Common Substructures

Bond	Abs. (approx., nm)	Transition
C=O (Ketone)	185-280	n→π* or π→π*
C=O (Aldehyde)	293	n→π*
C-O (MeOH)	183	n→σ*
RHC=CHR	165-190	π→π*
H2C=C-C=CH2	200-220	π→π*

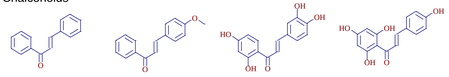
RESULTS

Compound Structure and UV Absorption

We studied the UVPD of two categories of compounds – phenylpropanoids and chalconoids. Chalconoids are precursors to the diverse chemical space of flavonoids and are themselves formed from coenzyme A thioesthers of phenylpropanoid derivatives. Phenylpropanoids themselves are almost ubiquitious in the plant kingdom and play a role in multiple parts of plant life including structural polymers, cell signaling, and plant defense.

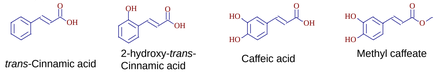
Figure 2. Phenylpropanoid and Chalconoid Structures

Chalconoids



Chalcone | 4-methoxychalcone | Butein | Naringeninchalcone

Phenylpropanoids



trans-Cinnamic acid | 2-hydroxy-*trans*-Cinnamic acid | Caffeic acid | Methyl caffeate

The phenylpropanoids, in addition to their chemical diversity through multiple hydroxy and methoxy isomers, also undergo various conjugations with sugars and other small molecules to create biologically active derivatives. Two such compounds, *trans*-Clovamide and chlorogenic acid (Figure 3) were also used in this study to investigate the potentially unique and diagnostic fragments available using UVPD for these compounds.

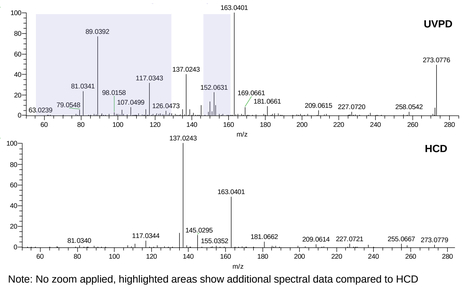
Figure 3. Phenylpropanoid Conjugates

trans-Clovamide | Chlorogenic acid

Phenylpropanoid and Chalconoid Behavior

When subjected to UVPD, all chalconoids displayed similar behavior in which the formation of fragment ions which typically require very high HCD energies were immediately observed in UVPD spectra with as few as 50 msec laser time. As a result, UVPD fragmentation spectra were typically richer than comparable HCD spectra (Figure 4, comparison of butein).

Figure 4. Butein Fragmentation: HCD (27eV) vs UVPD (150 msec)



Note: No zoom applied, highlighted areas show additional spectral data compared to HCD

A significant difference between the fragmentation approaches arises from the means in which they initiate fragmentation. In HCD, energy is imparted by the initial injection of the ions into the collision cell and collisions with a relatively static gas. A greater voltage offset gives rise to more energetic collisions. The energy is internally distributed with bonds breaking to form fragment ions which may also undergo subsequent fragmentation events generating several generations of fragment ions in a single scan. In UVPD, subsequent pulses may impart additional energy to fragments formed in previous pulses and create multi-generation fragments that can reach farther down into fragment pathways (Figure 5).

Figure 5. Comparing Kinetics: Methyl Caffeate – UVPD 50 msec vs. HCD Low and High eV

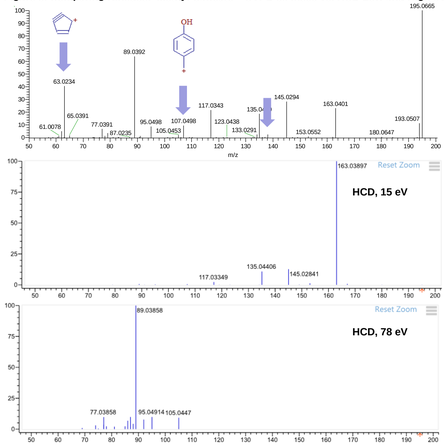
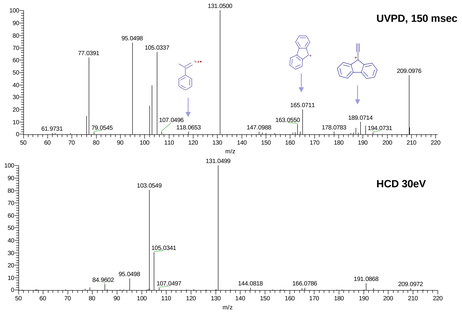


Figure 6. Fragmentation of Chalcone by UVPD – Kinetic Effects

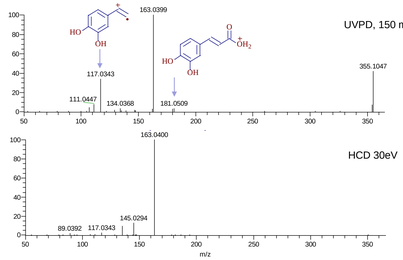


In addition to early observation of typically higher energy fragmentation channels in the UVPD, an increase in fragment ions arising from ionization of the aromatic rings or the conjugated double bond chalconoids was observed (Figure 6). While ionization was largely the result of the ketone or alcohol functions present on the compounds, specific absorption of photons generated unique fragmentation. Several of these fragment ions were not observed in HCD at any energy level (from 10 to 200% NCE).

Phenylpropanoid Conjugate Fragmentation

One key point of interest for UVPD is the potential generation of novel and unique fragmentation which could be diagnostic for structure determination of unknown compounds. In this work, special attention was given to the phenylpropanoid conjugates as additional fragmentation information could prove useful in determining structure especially when normal collision induced methods primarily result in the generation of the aconjugate fragment ion. In the case of chlorogenic acid, the initiation of fragmentation from the aromatic phenyl and conjugated double bond gave rise to fragment ions not observed in HCD at any energy level (Figure 7).

Figure 7. Fragmentation of Chlorogenic Acid – UVPD vs. HCD



CONCLUSIONS

- UVPD provided unique fragmentation for the compounds studied which may be useful for structure determination / substructure identification.
- The different mechanism of ion excitation and resulting kinetics of fragmentation for UVPD resulted in fragmentation spectra that were often richer than corresponding HCD fragmentation spectra.
- "Tuneable" laser energy (wavelength) or increased power may provide further utility with the ability to more selectively fragment molecules by targeting specific substructures.
- Further studies with differing wavelengths may provide even more diagnostic fragments.

TRADEMARKS/LICENSING