Using Parent Ion Conformations to Gain Insight into Fragmentation Mechanisms

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Overview

- Angiotensin II (Ang II), DRYXHHP, was reacted with ozone resulting in the formation of a variety of oxidation products (M+H). These products include Ang II, Tyr methylation, M+H-2, and M+H-3 modifications to Tyr and His.

- SID was used in a 6T FT-ICR MS to generate energy-resolved fragmentation efficiency curves (FERC) to compare charge-remote vs. charge-directed dissociation pathways.

- Molecular dynamics calculations were conducted to probe parent ion conformations to gain insight into the mechanistic characteristics of the fragmentation channels.

- Ang II O oxidation leads to new charge-remote and charge-directed pathways.
  - Charge-remote channels are observed in both the M+2 and M+3 oxidized products with mechanisms similar to Asp cleavage in unmodified Ang II.
  - Selective charge-directed dissociation pathways occur due to oxidation at lower onset energies than traditional non-selective backbone cleavages suggesting charge is easily transferred from Arg to the modified residue.

Introduction

Ozone is an abundant and vital compound in the environment with particular importance in atmospheric chemistry. It’s oxidizing properties and strong reactivity makes ozone extremely reactive with biomolecules. More specifically, prolonged exposure has been linked to adverse health effects as a result of oxidation of proteins and lipids in vivo. Previous studies have demonstrated that ozone reacts with Met, His, Cys, Phe, Tyr and Trp when present in small peptides (2-4 residues). Although the oxidation of peptides and proteins has been extensively studied little is known about the mechanism of oxidation due to ozonolysis and even less is known about the effect of oxygen addition on fragmentation patterns of oxidized peptides in MS/MS experiments. Ozone is a disposable modifier of Ang II (M+H) and other peptides by adding two oxygen atoms to form four primary oxidation products. Ang II needs to be modified with SID experiments, using FT-ICR MS, off the singly charged species verified that the primary oxidation products were due to the addition of 4 oxygen atoms to Tyr (1062,1631,1746 m/z). Oxygen atoms to His (1063,1730,2357 m/z), and an oxygen atom from the oxidation of both Tyr and His (1115,5230 m/z). Ang I, as previous experiments suggest, selectively fragment to form the Y fragment, while the M+2 product was shown to consist of 1115,5230 m/z due to the oxidized Tyr residue. Consequences, as seen in the M+3 product, leads to the selective formation of the b12 ion. Analysis of the parent ion survival curves using the RIFM approach and analysis of some of the M+3 modification pathways suggest charge-directed and dissociation. The goal of this study is to use molecular dynamics to probe parent ion conformations to gain insight into the dissociation mechanisms.

Methods

- EMSL FT-ICR Mass Spectrometer
- SID reactions were conducted with ozone (1000 ppm) for approximately 20 minutes.
- Analysis was done using a 6T FT-ICR 500 mass spectrometer.
- Collision energy-resolved SID data was obtained for each mass selected species with reaction time of 1 s.
- Molecular dynamics modeling was done using the Discover module of the Insight II software suite (Biosym Tech, San Diego, CA, USA).
- Steepness deficient minimization (1500 iterations) was done using the CPMD local code.
- Conformations were then annealed for 100,000 cycles (100ps) at 400K.

Conclusions

- Energy-resolved fragmentation efficiency curves distinguish between charge-remote vs. charge-directed dissociation pathways.
- Parent ion conformations, calculated using molecular dynamics, provided mechanistic characteristics of the selective fragmentation channels.
- Unmodified Ang II was shown to selectively cleave to form the y fragment via energy-resolved FERC confirming previous results.4,5
- Molecular dynamics calculations demonstrate the charge-remote nature of the y, mechanism.
- M+2 product Energy-resolved FERC data suggests that the b4 selective pathway is a charge-remote process.
- Analysis of the parent ion conformations of the tyrosine shows an additional tyrosine group interacts with the carbonyl of the backbone, leading to the Tyr residue resulting in the formation of the b12 ion.
- M+3 product Energy-resolved FERC data suggest that both charge-remote and charge-directed selective fragmentation channels are opened with the oxidation of the His residue.
- Molecular dynamics was used to determine the charge-remote loss of 45mz and 71mz is driven by strong hydrogen bonding within the modified His side chain.
- Preferred conformations highlight the interactions necessary for the charge-directed formation of the b12 ion and the loss of 18mz to the parent ion.

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References


Figure Legends

Results & Discussion

Angiotensin II

- Energy-resolved FERC confirms the y, selective fragmentation pathway is the lowest energy dissociation channel for singly charged Ang II.
- Other fragmentation channels occur at higher onset energies via charge-directed pathways after proton transfer to the backbone as suggested by the remote proton model.

Angiotensin II + O: Tyr Oxidation

- Energy-resolved FERC shows that the selective formation of the b12 ion is the most favorable fragmentation channel.
- Cleavage to form the y fragment can be explained by a two step process involving loss of Tyr and H2O by mechanisms shown.
- Higher energy processes are non-selective charge-directed pathways due to charge transfer to the backbone from the oxidized Arg residue.

Conclusions & Discussion

- Energy-resolved FERC suggests both charge-remote and charge-directed selective dissociation channel involving the oxidized His residue.
- Low-voltage energy pathways include the formation of the M+3-45 and M+3-71 ions due to charge-remote fragmentation (Mechanisms 1 and 2).
- The selective formation of the b12 ion and M+3-88 and higher energy pathways where charge can be transferred to the modified His residue resulting in charge-directed pathways (Mechanisms 3 and 4).
- Molecular dynamics suggest conformations that are consistent with the proposed mechanisms.
- Charge-remote dissociation pathways:
  - Proteolysis Arg residue is essential in the preferred conformations.
  - Figures C and D show strong hydrogen bonding interactions between the aromatic sidechain oxygen and the aromatic ring of the His side chain for the formation of the M+3-45 and M+3-71 respectively.
- Charge-directed dissociation pathways:
  - With the charge of His in the modified His side chain, preferred conformations show hydrogen bonding interaction consistent with the fragmentation by the remote formation of the b12 ion (Figure E).
  - Figure F displays conformations that provide a suitable route for the formation of the M+3-88 ion.