Using Parent Ion Conformations to Gain Insight into Fragmentation Mechanisms

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Overview

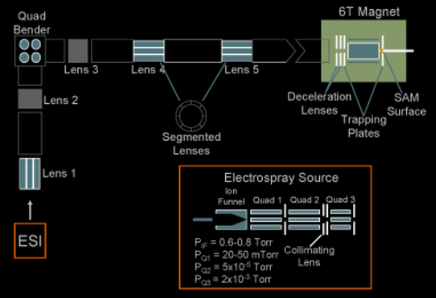
- Angiotensin II (AngII), DRVYIHPF, was reacted with ozone resulting in the formation of a variety of oxidation products (M+nO).
  - Primary products include M+O: Tyr modification, M+3O: His modification and M+4O: modification to Tyr and His.
- SID was done using a 6T FT-ICR MS to generate energy-resolved fragmentation efficiency curves (FEC) 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.
- AngII Oxidation leads to new charge-remote and charge-directed pathways.
  - Charge-remote channels are observed in both the M+O and M+3O products with mechanisms similar to Asp cleavage in unmodified AngII.
  - Selective charge-directed dissociation pathways occur due to His oxidation at lower onset energies than traditional non-selective backbone cleavages suggesting charge is easily transferred from Arg to the modified His 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 ozonation of peptides and proteins in living cells. Previous studies have demonstrated that ozone reacts with Met, His, Cys, Phe, Trp and Tyr when present in small peptides (2-4 residues).[1] Although the oxidation of peptides 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. Ozonolysis of Angiotensin II (1046.5423 m/z) has previously shown to form four primary oxidation products.[2] Accurate mass measurements and SID experiments, using FT-ICR MS, of the singly charged species verified that the primary oxidation products were due to the addition of 1 oxygen atom to Tyr (1062.5372 m/z), 3 oxygen atoms to His (1094.5270 m/z), and 4 oxygen atoms resulting from the oxidation of both Tyr and His (1110.5220 m/z).[2] Angiotensin II, as previous experiments suggest, selectively fragments to form the y_7 fragment, while the M+O adduct was shown to selectively form the b_4+O ion due to the oxidized Tyr residue. Oxidation of His, as seen in the M+3O product, leads to the selective formation of the b_5 ion. Analysis of the parent ion survival curves using the RRKM approach suggests that some of the new M+3O fragmentation channels have charge-directed characteristics. 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



- Flowing Ozone was reacted with the peptide solutions for approximately twenty minutes.
- Analysis was done using a 6T FT-ICR SID mass spectrometer.[3]
  - Collision energy resolved SID data was obtained for each mass selected oxidized species for 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).
  - Steepest descent minimization (1500 iterations) was done using the CFF91 force field.
  - Minimized conformations were then annealed for 100,000 cycles (100ps) at 400K.

Conclusions

- Energy-resolved fragmentation efficiency curves distinguished 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_7 fragment via Energy-resolved FEC confirming previous results.[4,5]
  - Molecular dynamics calculations demonstrate the charge-remote nature of the y_7 mechanism.
- ***M+O adduct*** Energy-resolved FEC data suggest that the b4 selective pathway is a charge-remote process.
  - Analysis of the parent ion conformations implies the hydrogen atom of the additional hydroxy group interacts with the carbonyl of the backbone C-term to the Tyr residue resulting in the formation of the b_4 ion.
- ***M+3O product*** Energy-resolved FEC results show 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 45m/z and 71m/z 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 b_5 ion and the loss of 88m/z to the parent ion.

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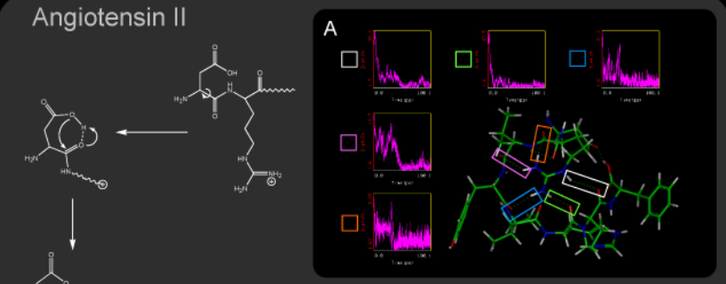
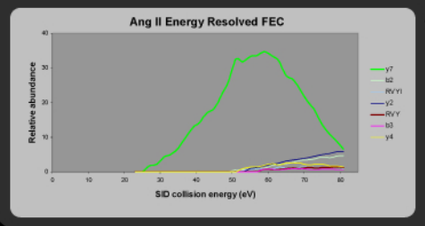
Results & Discussion

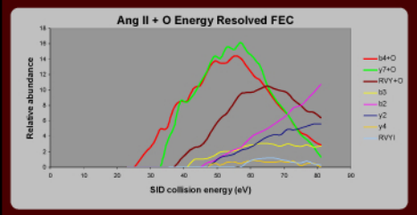
Angiotensin II



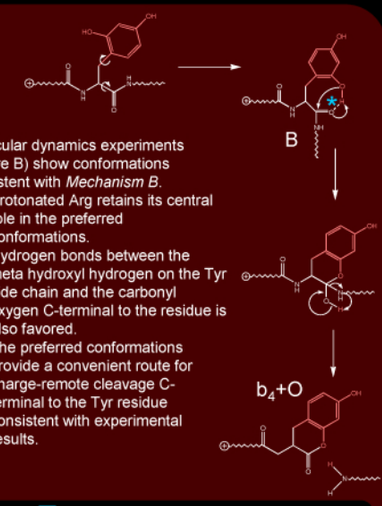
- Energy-resolved FEC confirms the y_7 selective fragmentation pathway is the lowest energy dissociation channel for singly charged Ang II.[4]
  - Other fragmentation channels occur at higher onset energies via charge-directed pathways after proton transfer to the backbone as suggested by the mobile proton model.[5]
- Molecular dynamics shows preferred conformations are characterized by hydrogen bonding between backbone carbonyls and the amide nitrogen atoms of the protonated Arg side chain (Figure A).
  - Suggests the y_7 fragmentation pathway results from charge-remote cleavage C-terminal to the Asp residue.
- Extensive charge solvation with the backbone demonstrates a reasonable route for proton transfer to occur.



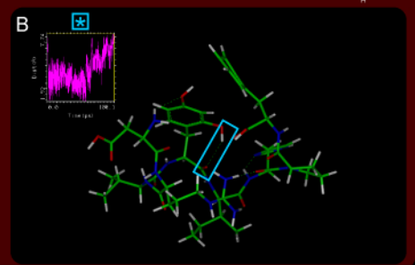
Angiotensin II + O: Tyr Oxidation

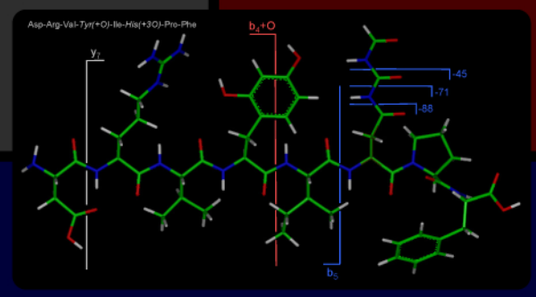


- Energy-resolved FEC shows that the selective formation of the b_4+O ion is the most favorable fragmentation channel.
  - Cleavage to form the RVY+O ion can be explained as a two step process involving both the b_4+O and y_7 mechanisms shown.
  - Higher energy processes are non-selective charge-directed pathways due to charge transfer to the backbone from the protonated Arg residue.

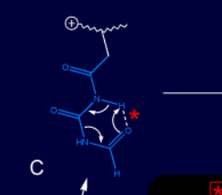


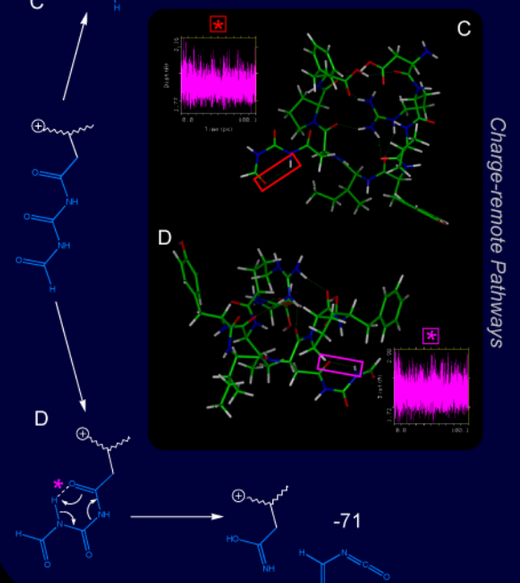
- Molecular dynamics experiments (Figure B) show conformations consistent with *Mechanism B*.
  - Protonated Arg retains its central role in the preferred conformations.
  - Hydrogen bonds between the meta hydroxyl hydrogen on the Tyr side chain and the carbonyl oxygen C-terminal to the residue is also favored.
  - The preferred conformations provide a convenient route for charge-remote cleavage C-terminal to the Tyr residue consistent with experimental results.





Angiotensin II + 3O: His Oxidation





- Energy-resolved FEC suggests both charge-remote and charge-directed selective dissociation channels involving the oxidized His residue.
  - Lowest energy pathways include the formation of the (M+3O)-45 and (M+3O)-71 ions due to charge-remote mechanisms (*Mechanisms C and D*).
  - The selective formation of the b_5 and (M+3O)-88 ions occur at higher onset energies where charge can be transferred to the modified His residue resulting in charge-directed pathways (*Mechanisms E and F*).
- Molecular dynamics show preferred conformations that are consistent with the proposed mechanisms.
  - *Charge-remote dissociation pathways*
    - Protonated Arg retains its central role in the preferred conformations.
    - *Figures C* and *D* show strong hydrogen bonding interactions between the appropriate carbonyl oxygen and the amide nitrogen of the His side chain for the formation of the (M+3O)-45 and (M+3O)-71 respectively.
  - *Charge-directed dissociation pathways*
    - With the charge shifted to the modified His side chain, preferred conformations show hydrogen bonding interaction consistent with the mechanism proposed for the selective formation of the b_5 ion (*Figure E*).
    - *Figure F* displays conformations that provide a suitable route for the formation of the (M+3O)-88 ion.