Ozonated Peptides: Insight into Structure and Fragmentation Energetics using SID FT-ICR MS

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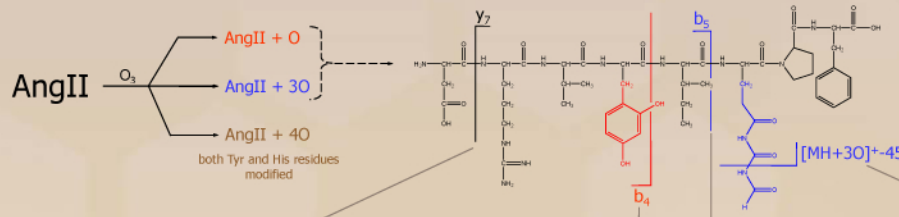
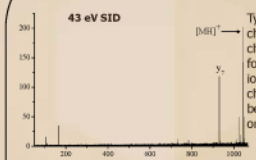
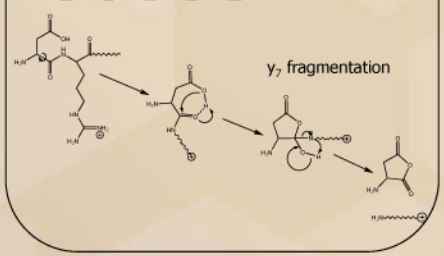
Results



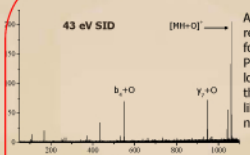
AngII Unmodified (1046 m/z)



Typical fragmentation characteristics of singly charged Ang II is the selective formation of the y7 fragment ion. This proceeds via a charge-remote mechanism because the charge is solvated on the Asp residue.[5]



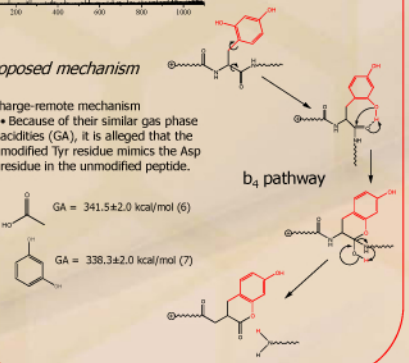
AngII + O Adduct (1062 m/z)



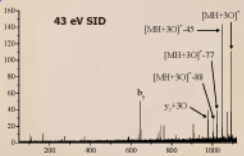
Addition of oxygen to the Tyr residue promotes selective formation of the b_4 fragment ion. Presumably, the charge is still localized on the Arg residue and the modified Tyr residue is acting like the Asp residue does under normal circumstances.

Proposed mechanism

- Charge-remote mechanism
  - Because of their similar gas phase acidities (GA), it is alleged that the modified Tyr residue mimics the Asp residue in the unmodified peptide.



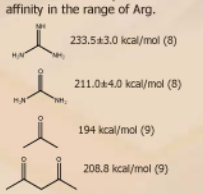
AngII +3O Adduct (1094 m/z)



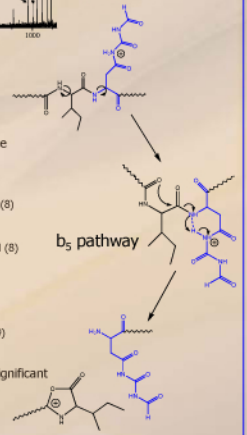
Addition of 3 oxygen atoms to the His residue results in multiple losses to the modified side chain and the selective formation of the b_5 ion upon fragmentation. This suggests the charge is localized on the modified His residue.

Proposed Mechanisms

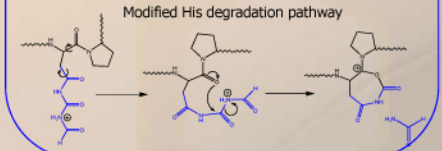
- Charge-directed mechanisms
  - Comparison of model compounds suggests that the modified His has a proton affinity in the range of Arg.



- Acetylation results in a significant increase in proton affinity.

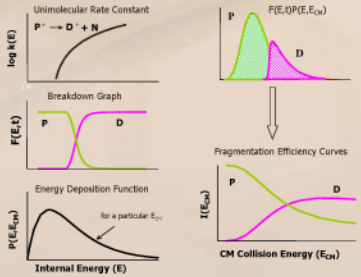


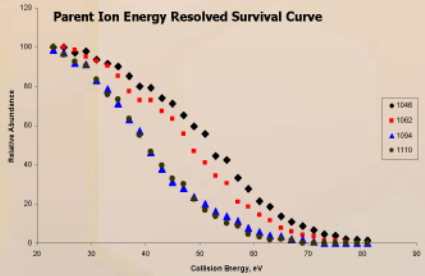
Modified His degradation pathway



Ion Energetics

We collect mass spectra at different collision energies and reaction times and model them using RRKM





Results of RRKM modeling of the parent ion survival curves

[MH+nO]+	n=0	n=1	n=3	n=4
M/Z	1046	1062	1110	1126
E_o (eV)	1.14	1.20	1.21	1.24
$\Delta S^{\ddagger}$ (cal/mol K)	-25.9	-21.6	-17	-15.3
Relative E_o	0	0.06	0.07	0.11
A, s^{-1}	5.6E+07	4.8E+08	4.8E+09	1.2E+10
Log (A)	7.7	8.7	9.7	10.1
T-V %	18.1			
k_{rad} (s^{-1})	55			

E_o is the threshold energy, $\Delta S^{\ddagger}$ is the entropy change for the transition state at 450 K, A is the pre-exponential factor at 450 K, T-V % is the percentage of the ion's kinetic energy converted to internal energy upon collision, and k_{rad} is the radiative decay rate.

- Data Interpretation
  - Addition of oxygen by ozonolysis destabilizes the parent ion.
    - Caused by the opening of additional selective fragmentation pathways.
  - The shift to lower collision energies is entirely due to the entropy effect (increasing pre-exponential factor).
    - E_o increases slightly with oxidation
    - $\Delta S^{\ddagger}$ increases with oxidation
      - New fragmentation pathways have "looser" transition states
    - The dramatic shift with addition of 3 and 4 oxygen atoms may result from a shift from charge-remote to charge-directed fragmentation pathways.

Overview

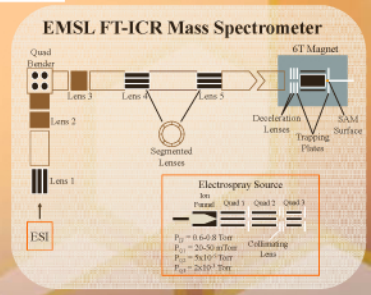
- Angiotensin II (AngII) was reacted with ozone resulting in the formation of a variety of oxygen addition products (M+nO).
- FT-ICR-MS SID was used to obtain energy resolved fragmentation data to gain insight into the differences in fragmentation characteristics of singly charged AngII and each of the singly charged oxygen adducts.
- Information regarding structure, fragmentation mechanisms and energetics was acquired.

Introduction

Ozone is an abundant and vital compound in the environment with particular importance in atmospheric chemistry. Its 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 the 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. The goal of this study is to understand the fragmentation mechanisms and energetics of the ozonolysis products of Angiotensin II. AngII was chosen to begin our studies because it contains two amino acids that are known to react with ozone and provides an opportunity to examine the effects of ozonolysis on slightly larger peptides.

Method

- Flowing Ozone was reacted with the peptide solutions for approximately twenty minutes.
- Analysis was done using a 6T FT-ICR SID mass spectrometer[2]
- Collision energy resolved SID data was obtained for each mass selected oxidized species for reaction time of 1 s.
- Energy resolved survival curves were generated and analyzed using an RRKM approach (see results) developed previously.[3,4]



Conclusions

- Accurate mass measurement was used to verify that the oxidation products were due to the addition of 1 oxygen atom (1062.5391 m/z), 3 oxygen atoms (1094.5362 m/z), and 4 oxygen atoms (1110.4813 m/z)
- $[MH+O]^+$ selectively fragments to form the b4+O ion due to oxidation to the Tyr residue
  - Modified Tyr acts like Asp allowing for charge-remote mechanisms, suggesting that oxidation takes place in the meta position relative to the Tyr hydroxyl group.
- $[MH+3O]^+$ fragments mainly via charge-directed fragmentation pathways induced by the oxidized His residue
  - Both His degradation pathways and the formation of the b5 ion suggest that the oxidized His residue competes for charge with Arg
- $[MH+4O]^+$ shows fragmentation properties of both the $[MH+3O]^+$ and $[MH+4O]^+$ adducts
- Parent ion survival curves were analyzed using an RRKM approach
  - Shifts of survival curves to lower collision energies result from increase in reaction entropy
  - Charge-directed dissociation pathways induced by oxidized His are associated with more positive entropy effects than charge-remote fragmentation

Acknowledgements

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References

(1) Kotiaho, T.; Eberlin, M.N.; Vainiotalo, P.; Kostiainen, R. *J. Am. Soc. Mass Spectrom.* 2000; 11, 526-35.
(2) Laskin Julia; Denisov Eduard V.; Shukla Anil K.; Barlow Stephan E.; Futrell Jean H. *Anal. Chem.* 2002; 74, 3255-61.
(3) Laskin, Julia; Byrd, Michelle; Futrell, Jean. *Int. J. Mass Spectrom.* 2000; 195/196, 285-302.
(4) Laskin, Julia; Futrell, Jean. *J. Phys. Chem. A* 2000; 104, 5484-94.
(5) Gu, C.; Tsaprailis, G.; Breci, L. A.; Wysocki, Vicki H. *Anal. Chem.* 2000; 72, 5804-13.
(6) Cumming, J.B.; Kebarle, P. *Can. J. Chem.* 1978; 56, 1-9.
(7) Kebarle, P.; McMahon, T.B., *J. Am. Chem. Soc.* 1977; 99, 2222-30.
(8) Kinser, R. D. *Ph.D. Dissertation, The University of Delaware*, 1993; 143-4.
(9) Hunter, E.P.; Lias, S.G. *J. Phys. Chem. Ref. Data* 1998; 27, 3, 413-656.