Gas-Phase Structure and Unimolecular Dissociation of Cysteine Sulfinyl Radical Ions

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Overview

- Tandem mass spectrometry and *ab initio* calculations were used to probe the structure and understand gas-phase unimolecular dissociation behavior of small cysteine containing sulfinyl radical ions.
- N-acetylation, O-methylation, stable-isotope labeling were used to investigate the structural and energetic information.
- Location of charge significantly impacts charge vs. radical driven fragmentation of sulfinyl radical ions.
- Proposed mechanism for the major fragmentation pathways are presented.

Introduction

- Radicals play important roles in biological systems via reactions toward a wide variety of biomolecules. Undesirable chemical modification of biomolecules by OH radical can result in irreversible cell damage or lysis. Irregular concentrations of hydroxyl radical has proven to be related to oxidative stress and aging.[1-2]
- Sulfur containing amino acid residues, such as cysteine and methionine, are among the most reactive sites toward OH attack.[3]
- Characterization of the thus formed peptide/protein radical intermediates is a key step to understanding the associated biological consequences.[4]
- Insight on distonic ions as reactive intermediates for unimolecular mass spectrometric fragmentation.[5]
- In this presentation, gas-phase cysteine sulfinyl radical ions (cations) were formed via oxidative cleavage of disulfide bond within cystine or modified cystine ions.

Methods

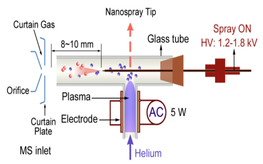


Figure 1. Schematic view of the experimental setup

- NanoESI for peptide ion formation
- Atmospheric pressure helium low temperature plasma (LTP) used for hydroxyl radical formation.[6]
- The interactions between hydroxyl radicals and peptide ions were facilitated in a glass flow tube as shown in Figure 1.
- A 4000Qtrap mass spectrometer was used for data collection.
- All peptides were prepared in 50/49/1 MeOH/H_2O/HOAc (v/v/v) with a final concentration of 10 µM.
- Deuterated peptides were prepared as 99:1 (v/v) D_2O/acetic acid solutions.

Results and Discussion

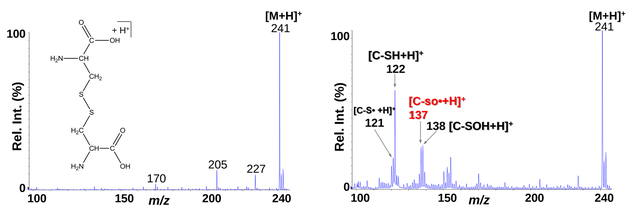
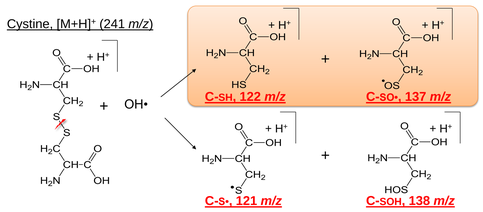


Figure 2. NanoESI MS^1 of protonated cystine before (left) and after (right) application of the LTP source on.



Beam-type CID of Cysteinyl sulfinyl radical ions

Cysteine sulfinyl radical ions

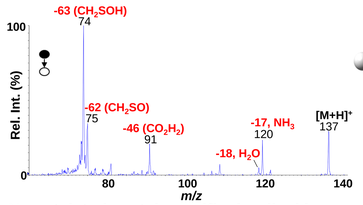


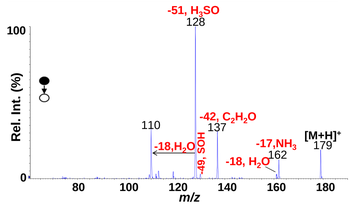
Table 1. Spin densities for cysteine sulfinyl radical at various protonation sites.

Cys-SO•	Spin densities
Neutral	S: 0.510 O: 0.488
Protonated at NH_2	S: 0.543 O: 0.456
Protonated at C=O	S: 0.552 O: 0.453

Table 2. Comparable energy differences for protonated structures.

Protonation	Energy
C=O	+112.9 kJ/mol
CH_2	+144.4 kJ/mol
SO	+2.63x10^4 kJ/,mol

N-acetylated cysteine sulfinyl radical ions



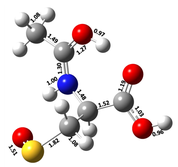


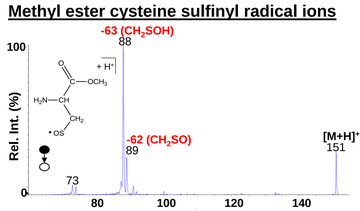
Table 3. Spin densities for N-acetylated cysteine sulfinyl radical at various protonation sites.

N-ACys-SO•	Spin densities
Protonated at C=O acetyl	S: 0.425 O: 0.602
Protonated at C=O carboxylic	S: 0.395 O: 0.626
Protonated at S-O●	S: 0.913 O: 0.134

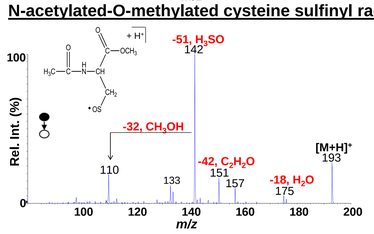
Table 4. Comparable energy differences for protonated structures.

Protonation	Energy
C=O carboxylic	+29.5 kJ/mol
SO	+55.1 kJ/mol
NH	+68.9 kJ/mol

Methyl ester cysteine sulfinyl radical ions



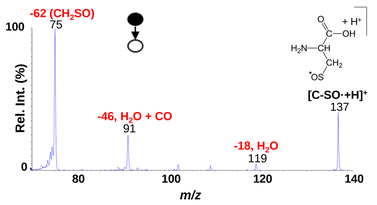
N-acetylated-O-methylated cysteine sulfinyl radical ions

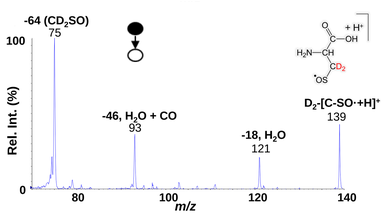


- **Location of charge significantly impacts charge vs. radical driven fragmentation of sulfinyl radicals**
- **Cysteine sulfinyl radical resulted in radical driven 62Da loss (CH_2OH) as major fragmentation pathway**
- **However, N-acetylated cysteine sulfinyl radical resulted in the major fragmentation channel of a charge driven 51Da loss**

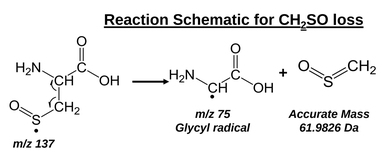
Mechanism for 62Da loss (CH_2SO)

Ion trap CID of cysteine and D2-cystiene sulfinyl radical ions

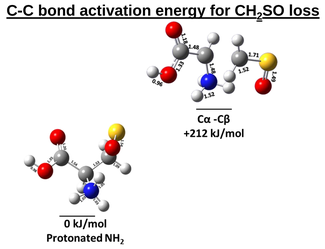




Reaction Schematic for CH_2SO loss



C-C bond activation energy for CH_2SO loss



Conclusions

- Location of charge plays a role in charge driven vs. radical driven fragmentation of cysteine sulfinyl radical ions
- Protonation on nitrogen resulted in 62Da loss (CH_2SO)
- Formation of glycl radical
- Dominant 51Da product ion loss (H_3SO) when cystienyl sulfinyl radical ions are acetylated
- Loss of H_3SO is sequential loss from the initial H_2O loss
- Mobile proton, beta carbon proton, acetyl nitrogen hydrogen are involved in H_3SO loss

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5. Gruetmacher, H. F., *Int. J. Mass Spectrom. Ion Processes,* **1992**, 118/119, 825-55.
6. Ma, X., Love, C.B., Zhang, X., Xia, Y., *J. Am. Soc. Mass Spectrom.*, **2011**, 22, 922-930.

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- Purdue University Startup Fund
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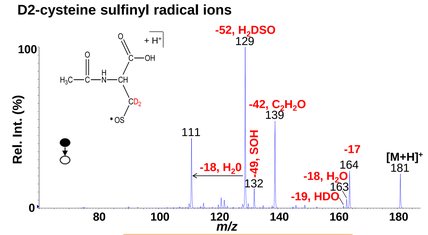
- Dr. Graham Cook's research group at Purdue for accurate mass measurements data

51Da loss (H_3SO)

Accurate mass: 50.9904Da Theoretical mass: 50.9994Da

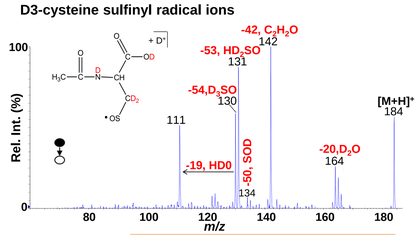
Isotopic Deuterated Labeling

D2-cysteine sulfinyl radical ions



One Hydrogen comes from beta carbon

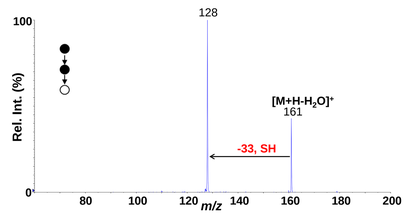
D3-cysteine sulfinyl radical ions



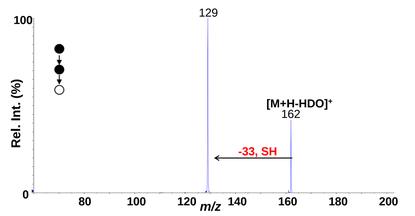
Another Hydrogen comes from acetyl nitrogen

MS^3 CID of H_2O loss

N-acetylated cysteine sulfinyl radical ions



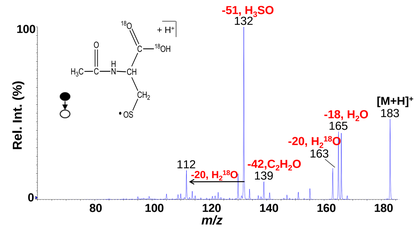
D2-cysteine sulfinyl radical ions



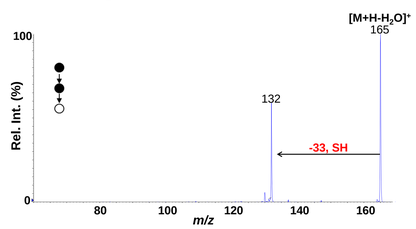
CID of H_2O/ HDO shows consecutive loss of 33Da (SH)

^{18}O labeled experiments

18O-N-acetyled cysteine sulfinyl radical ions



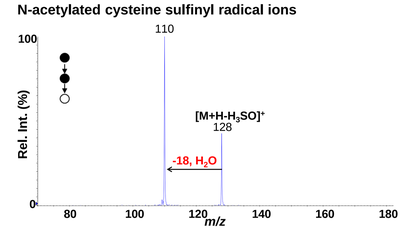
MS^3 CID of H_2O loss



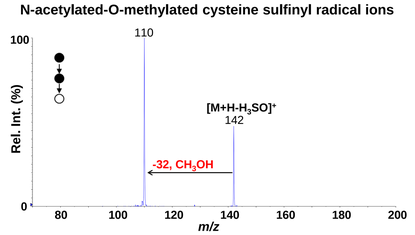
MS^3 of H_2O loss resulting in 33Da loss (SH) gives evidence that carboxyl oxygen are not involved with H_3SO loss

MS^3 CID of H_3SO loss

N-acetylated cysteine sulfinyl radical ions



N-acetylated-O-methylated cysteine sulfinyl radical ions



CID of H_3SO shows carboxyl group (COOH, $COOCH_3$) is not involved with H_3SO loss

Possible Mechanism for H_3SO loss

Activation energy for H_2O loss