



Published in final edited form as:

*J Am Soc Mass Spectrom.* 2015 July ; 26(7): 1103–1114. doi:10.1007/s13361-015-1125-y.

## Transformation of $[M+2H]^{2+}$ Peptide Cations to $[M-H]^+$ , $[M+H+O]^+$ , and $M^{+\bullet}$ Cations via Ion/Ion Reactions: Reagent Anions Derived from Persulfate

Alice L. Pilo, Jiexun Bu, and Scott A. McLuckey\*

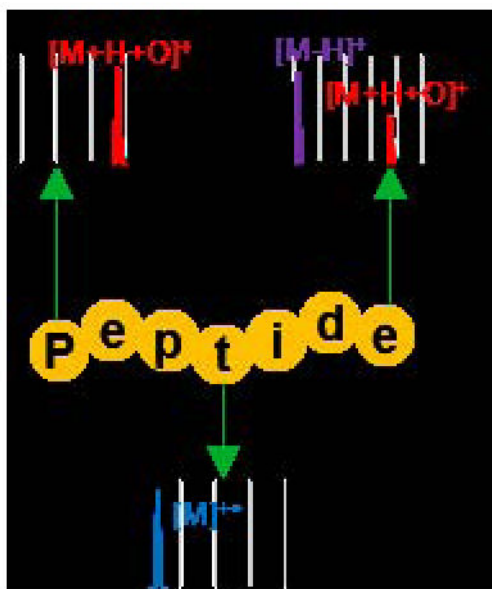
Department of Chemistry, Purdue University, West Lafayette, IN 47907-2084

### Abstract

The gas-phase oxidation of doubly protonated peptides is demonstrated here using ion/ion reactions with a suite of reagents derived from persulfate. Intact persulfate anion ( $HS_2O_8^-$ ), peroxymonosulfate anion ( $HSO_5^-$ ), and sulfate radical anions ( $SO_4^{\bullet-}$ ) are all either observed directly upon negative nanoelectrospray ionization (nESI) or easily obtained via beam-type collisional activation of persulfate into the mass spectrometer. Ion/ion reactions between each of these reagents and doubly protonated peptides results in formation of a long-lived complex. Collisional activation of the complex containing a peroxymonosulfate anion results in oxygen transfer from the reagent to the peptide to generate the  $[M+H+O]^+$  species. Activation of the complex containing intact persulfate anion either results in oxygen transfer to generate the  $[M+H+O]^+$  species or abstraction of two hydrogen atoms and a proton to generate the  $[M-H]^+$  species. Activation of the complex containing sulfate radical anion results in abstraction of one hydrogen atom and a proton to form the peptide radical cation,  $[M]^{+\bullet}$ . This suite of reagents allows for the facile transformation of the multiply protonated peptides obtained via nESI into a variety of oxidized species capable of providing complementary information about the sequence and structure of the peptide.

### Abstract

\*Address reprint requests to: Dr. S. A. McLuckey, 560 Oval Drive, Department of Chemistry, Purdue University, West Lafayette, IN 47907-2084, USA, Phone: (765) 494-5270, Fax: (765) 494-0239, mcluckey@purdue.edu.



## INTRODUCTION

Tandem mass spectrometry is a powerful approach for obtaining primary structural information about a bioanalyte of interest. The information obtained from a tandem mass spectrometry experiment is highly dependent on the nature of the gas-phase ions generated from the molecules of interest.<sup>1</sup> Different ion types (e.g., protonated molecule, deprotonated molecule, metallated molecule, radical cation, radical anion, etc.) undergo different fragmentation pathways upon activation, which can yield complementary information. It is therefore useful to be able to generate different types of ions and to manipulate ion-type within the mass spectrometer. The advent of electrospray ionization (ESI) has made the generation of multiply-charged analytes straightforward.<sup>2,3</sup> These ions are typically protonated in the positive polarity and deprotonated in the negative polarity. Gas-phase ion/ion reactions have been successful in transforming ESI-generated ions to an ion-type different from the type initially generated within a mass spectrometer.<sup>4</sup>

The reduction of charge via proton transfer,<sup>5,6</sup> electron transfer both to and from multiply-charged analytes,<sup>7,8</sup> and the addition or removal of metal ions<sup>9,10,11</sup> are examples of the manipulation of ion-type in the gas-phase via ion/ion reactions. Site-selective covalent modification of peptides and proteins has also been demonstrated via ion/ion reactions. For example, N-hydroxysuccinimide (NHS) esters have been used to cross-link<sup>12,13</sup> and covalently label<sup>14,15,16</sup> various nucleophiles in peptide ions, 4-formyl-1,3-benzenedisulfonic acid (FBDSA) has been used to tag peptide ions via Schiff base chemistry,<sup>17,18,19</sup> and *N*-cyclohexyl-*N'*-(2-morpholinoethyl)carbodiimide (CMC) has been used to selectively react with carboxylic acids<sup>20</sup> in various analytes. Ion/ion reactions occur on the typical reaction time-scale of ~100 ms and can either proceed via the long-range transfer of small charged particles, e.g., protons or electrons, or through the formation of a long-lived complex.<sup>4</sup> This gas-phase approach, particularly when implemented in a tandem mass spectrometer that enables the mass-selection of both the reagent and analyte ions, allows greater control of the

extent of modification relative to solution-phase derivatization, which can complicate the mass spectrum.

Recently, the oxidation of peptides via ion/ion reactions has been described.<sup>21</sup> Peptides containing methionine and tryptophan are selectively oxidized to  $[M+H+O]^+$  cations upon ion/ion reactions with periodate anions. The oxidation of the methionine side-chain to the sulfoxide derivative induces a signature loss of 64 Da corresponding to the ejection of methanesulfenic acid ( $CH_3SOH$ ).<sup>22,23,24,25,26,27,28,29,30</sup> This signature loss can be used to determine the presence and location of a methionine residue. Additionally, strategies to oxidize protonated peptides to their radical analogs, viz.,  $[M]^{+\bullet}$ , via ion/ion reactions have recently been described.<sup>31</sup> The gas-phase chemistry of radical peptide ions is an area of growing interest as these species undergo different fragmentation pathways than their protonated analogs upon activation.<sup>32,33</sup> To this end, several strategies have been developed to generate radical cations in the gas-phase to be analyzed via mass spectrometry.<sup>32,33</sup> These strategies include collision-induced dissociation (CID) of nitrosopeptides,<sup>34,35</sup> CID of ternary metal-ligand-peptide complexes,<sup>36,37,38,39,40,41,42</sup> photolysis of peptides with iodinated tyrosine residues<sup>43</sup> or iodinated electrostatic complexes,<sup>44</sup> and free radical-initiated reactions.<sup>45,46</sup>

Here, we describe ion/ion reactions between peptide cations and three reagent anions derived from persulfate to form three oxidized species;  $[M+H+O]^+$ ,  $[M]^{+\bullet}$ , and  $[M-H]^+$ . The three reagents are intact persulfate anions ( $HS_2O_8^-$ , Supplemental Figure S-1(a)), peroxymonosulfate anions ( $HSO_5^-$ , Supplemental Figure S-1(b)), and sulfate radical anions ( $SO_4^{\bullet-}$ , Supplemental Figure S-1(c)). Reactions between doubly protonated peptides and each of the three reagents results in formation of a long-lived complex. Activation of ion/ion complexes with intact persulfate anions yields oxygen addition, viz.,  $[M+H+O]^+$ , and hydrogen deficient species, viz.,  $[M-H]^+$ . Activation of analogous complexes with peroxymonosulfate anions yield the oxygen addition product, viz.,  $[M+H+O]^+$ . The last reagent derived from persulfate is sulfate radical anion, produced via homolytic cleavage of the persulfate peroxy-bond, which is observed upon negative nESI of an aqueous solution of sodium persulfate (Supplemental Figure S-1(d)). Activation of the complex produced via ion/ion reactions with peptide dications and sulfate radical anions yields the molecular radical peptide cation,  $[M]^{+\bullet}$ . This suite of reagents derived from persulfate readily and efficiently converts protonated peptides into various oxidized forms that, upon activation, may yield additional information about peptide primary structure.

## EXPERIMENTAL SECTION

### Materials

Methanol and glacial acetic acid were purchased from Mallinckrodt (Phillipsburg, NJ, USA). Sodium persulfate, angiotensin III, and melittin were purchased from Sigma Aldrich (St. Louis, MO, USA). Substance P and bradykinin were synthesized by Bachem (King of Prussia, PA, USA). ARAAKA and ARAMAKA were synthesized by NeoBioLab (Cambridge, MA, USA). KGAILAGAILR and GAILAGAILR were synthesized by SynPep (Dublin, CA, USA) and GAGGMGAGGRL was synthesized by Pepnome Ltd. (Shenzhen, China). All peptide stock solutions for positive nanoelectrospray were prepared in a

49.5/49.5/1 (vol/vol/vol) solution of methanol/water/acetic acid at an initial concentration of ~1 mg/mL and diluted 100-fold prior to use. The persulfate solution was prepared in 18 MΩ purified water using a Nanopure ultrapure water system from Barnstead/ThermoFisher Corp (Dubuque, IA) at a concentration of ~1 mg/mL and diluted 10-fold prior to use.

### Mass Spectrometry

All experiments were performed on a QTRAP 4000 hybrid triple quadrupole/linear ion trap mass spectrometer (AB Sciex, Concord, ON, Canada), previously modified for ion/ion reactions.<sup>47</sup> Multiply protonated peptides were isolated in the Q1-mass filter and injected into the q2 reaction cell followed by singly charged reagent anions via alternately pulsed nano-electrospray (nESI).<sup>48</sup> Both the intact persulfate anion ( $\text{HS}_2\text{O}_8^-$ ) and sulfate radical anion species ( $\text{SO}_4^{\bullet-}$ ) were present upon negative nESI of aqueous persulfate and were isolated in Q1 prior to injection into the q2 reaction cell. Peroxymonosulfate anion ( $\text{HSO}_5^-$ ), however, is a fragment derived from intact persulfate and was obtained through beam-type CID into the instrument via energetic interface conditions prior to isolation and injection into q2. The peptide cations and reagent anions were allowed to react for a mutual storage reaction time of 20 ms – 1000 ms. The ion/ion reaction products were then transferred to Q3, where the complex was subjected to further characterization via  $\text{MS}^n$  and mass analysis using mass-selective axial ejection (MSAE).<sup>49</sup>

### Calculations

Density function theory calculations have been carried out using the Gaussian 09 package.<sup>50</sup> Structural optimizations and energy calculations were performed with unrestricted B3LYP at the 6-31G(d) basis set for  $\text{HSO}_4^-$ ,  $\text{SO}_4^{\bullet-}$ ,  $\text{CH}_3\text{COOH}$ , and  $\text{CH}_3\text{COO}^\bullet$ . The bond dissociation energy (BDE) for  $\text{H-SO}_4^-$  was calculated by the isodesmic reaction method<sup>51</sup> using  $\text{CH}_3\text{COO-H}$  as the reference molecule, the BDE of which was previously determined experimentally.<sup>52</sup> In detail,  $\text{BDE}(\text{H-SO}_4^-)$  was calculated using the following equation.

$$\text{BDE}(\text{H-SO}_4^-) = \text{BDE}(\text{H-SO}_4^-)_{\text{calculated}} + \text{BDE}(\text{CH}_3\text{COO-H})_{\text{calculated}} - \text{BDE}(\text{CH}_3\text{COO-H})_{\text{experimental}}$$

The value was then compared with amino acid BDEs reported by the Julian group calculated via the same method.<sup>53</sup>

## RESULTS AND DISCUSSION

### Ion/Ion Reactions with the Suite of Reagents Derived from Persulfate

The nESI spectrum of an aqueous solution of sodium persulfate is shown in Supplemental Figure S-1(d). The base peak is the sodiated persulfate anion, though protonated persulfate is also present in a high enough abundance for ion/ion reactions. While the sulfate radical anion is observed at the same mass-to-charge ratio as the persulfate dianion, the majority of this peak is the singly-charged radical species based on the isotopic distribution and lack of anionic products observed during charge-inversion experiments (e.g., the reaction of singly protonated peptides with dianions resulting in final negative charge overall). The doubly charged species cannot be separated from the sulfate radical anion species of interest on the

basis of mass-to-charge ratio alone. However, the only deleterious impact from the presence of a small persulfate dianion population in the sulfate radical anion population in reactions with doubly charged peptide cations is a slight decrease in overall peptide ion signal due to neutralization of the peptide by the dianion. While some peroxymonosulfate anion is present in the original nESI spectrum acquired using a nozzle-skimmer voltage difference of 25 V (Supplemental Figure S-1(d)), the abundance is significantly increased upon use of a nozzle-skimmer voltage difference of 75 V (Supplemental Figure S-1(e)). A separate MS/MS experiment indicated that CID of the singly charged persulfate anion results predominantly in formation of the peroxymonosulfate anion (data not shown).

Ion/ion reactions between doubly protonated peptides and each of the reagent anions derived from negative nESI of persulfate results in the formation of a long-lived complex. Activation of this complex can either result in proton transfer from the peptide to the reagent to generate the charge-reduced  $[M+H]^+$  species or undergo one of the oxidative pathways outlined in Scheme 1. The first two pathways (red arrows in Scheme 1) result in oxygen transfer to the peptide to form the  $[M+H+O]^+$  species. Pathway A occurs via  $H_2SO_4$  loss from either CID of complexes with peroxymonosulfate anions or  $MS^3$  of the  $SO_3$  loss from complexes with persulfate anions. Pathway B occurs via  $H_2SO_4$  loss followed by  $SO_3$  loss from complexes with persulfate anions. While Pathway B also generates an oxidized species of the form  $[M+H+O]^+$ , the structure of the final product is not necessarily the same as the one obtained via Pathway A, as they proceed through different intermediates. Loss of two sulfuric acid moieties from the ion/ion complex with persulfate also occurs and results in the hydrogen-deficient  $[M-H]^+$  species via Pathway C (blue arrow in Scheme 1). The formation of the peptide radical cation, viz.,  $[M]^{\bullet+}$ , takes place via Pathway D (orange arrow in Scheme 1), which involves doubly protonated peptide in reaction with the sulfate radical anion and subsequent CID of the complex to lose  $H_2SO_4$ . To demonstrate each of these pathways, doubly protonated substance P (RPKPQQFFGLM) was reacted with each of the reagents derived from persulfate ( $HS_2O_8^-$ ,  $HSO_5^-$ , and  $SO_4^{\bullet-}$ ) and the resulting ion/ion complexes were subjected to CID (Figure 1). Activation of the ion/ion complex between substance P and peroxymonosulfate anion proceeds exclusively via Pathway A to yield the  $[M+H+O]^+$  species (Figure 1(a)). Activation of the ion/ion complex between substance P and persulfate anion yields loss of  $SO_3$  and loss of  $H_2SO_4$  as well as formation of the oxidized  $[M+H+O]^+$  and  $[M-H]^+$  species (Figure 1(b)). The  $[M+H+O]^+$  species is produced via a combination of Pathways A and B whereas the  $[M-H]^+$  species is produced via Pathway C. Pathway D is illustrated in Figure 1(c) via CID of the complex produced upon ion/ion reaction of doubly protonated substance P with sulfate radical anion, which undergoes loss of  $H_2SO_4$  to yield the  $[M]^{\bullet+}$  cation. Each of the reagents derived from persulfate are discussed in detail below.

### **Ion/Ion Reactions with Peroxymonosulfate ( $HSO_5^-$ ) Anions to Form $[M+H+O]^+$ Species**

Ion/ion reactions with peroxymonosulfate anions result in oxidation of a peptide via oxygen transfer from the reagent to the peptide. While the selective oxidation of methionine and tryptophan side-chains via ion/ion reactions with periodate has recently been described,<sup>21</sup> peroxymonosulfate appears to be a stronger oxidizing reagent as peptides both containing and lacking methionine and tryptophan residues are oxidized. This is demonstrated in Figure

2 with ARAAKA, a peptide that does not contain methionine or tryptophan residues and, as such, is not susceptible to oxidation by periodate. The ion/ion reaction between doubly protonated ARAAKA and peroxymonosulfate results in both proton transfer from the peptide to the anionic reagent resulting in the charge-reduced  $[M+H]^+$  species and complex formation to produce the  $[M+2H+HSO_5^-]^+$  species (Figure 2(a)). Activation of the complex either results in generation of the charge-reduced species via proton transfer or formation of the oxidized  $[M+H+O]^+$  species via Pathway A (Figure 2(b)). Note that the extent of oxidation in this case is much lower than for substance P (Figure 1(a)), which has a methionine residue at the C-terminus. A proposed mechanism for peroxymonosulfate oxidation is outlined in Scheme 2, which is analogous to the accepted mechanism for oxygen transfer from alkyl hydrogen peroxides.<sup>54</sup> The peroxymonosulfate anion first abstracts a proton from the peptide. One of the nitrogen atoms on the arginine side-chain then initiates a nucleophilic attack on the distal peroxy oxygen atom. Rearrangement to lose neutral  $H_2SO_4$  occurs and yields a charge-separated  $[M+H+O]^+$  species. Further rearrangement to eject  $NH_2OH$  (33 Da) can proceed via abstraction of a proton by the negatively charged oxygen atom to yield the final species shown in Scheme 2. While arginine was chosen to illustrate the proposed mechanism in Scheme 2, other sites on the peptide are capable of being oxidized via similar mechanisms as well (Supplemental Figure S-2).

Activation of the oxidized species results in formation of an abundant  $b_6+O$  ion (Figure 2(c)), which is produced via cleavage C-terminal to the lysine residue. This spectrum is similar to CID of the unmodified peptide in which the  $b_6$  ion is the dominant fragment observed (Supplemental Figure S-3). Further activation of the  $b_6+O$  ion was performed to gather more information about the site of oxidation, as shown in Figure 2(d). There are 33 Da losses present throughout the product spectrum likely corresponding to losses of  $NH_2OH$  from oxidized lysine or arginine side-chains (Scheme 2). Additionally, while a series of oxidized b-ions are observed, the presence of both the oxidized and the non-oxidized  $b_2$ -ion indicate the likelihood of multiple sites within the peptide being oxidized upon ion/ion reaction with peroxymonosulfate anions.

While activation of the ion/ion complex between doubly protonated ARAAKA and peroxymonosulfate anion resulted in mainly proton transfer to produce the charge-reduced species with oxidation of the peptide observed as a minor pathway (Figure 2(b)), activation of the analogous complex with ARAMAKA exclusively results in oxygen atom transfer (Figure 3(a)). Additionally, activation of the oxidized ARAMAKA species,  $[M+H+O]^+$ , yields dominant 64 Da losses corresponding to ejection of  $CH_3SOH$  from the oxidized methionine side-chain (Figure 3(b)).<sup>22-30</sup> While the presence of a methionine or tryptophan residue is not necessary for oxidation to occur, peptides containing these residues demonstrate increased complex formation and oxidation efficiencies and undergo preferential oxidation of the methionine or tryptophan side-chain. The peroxymonosulfate anion oxidation chemistry is also demonstrated with bradykinin, a widely studied 9-residue peptide (RPPGFSPFR). The efficiency of oxidation with bradykinin is ~10%, which is similar to that observed with ARAAKA (compare Figure 3(c) with Figure 2(b)). Additionally, similarly to ARAAKA, the presence of both oxidized and non-oxidized  $y_8$  ions upon CID of the  $[M+H+O]^+$  species indicates the presence of multiple oxidation sites in

the peptide (Figure 3(d)). Spectra illustrating the reactions between  $\text{HSO}_5^-$  and the  $[\text{M}+2\text{H}]^{2+}$  species of ARAMAKA and bradykinin are shown in Supplemental Figure S-4.

### **Ion/Ion Reactions with Intact Persulfate ( $\text{HS}_2\text{O}_8^-$ ) Anions to Form $[\text{M}+\text{H}+\text{O}]^+$ and $[\text{M}-\text{H}]^+$ Species**

Intact persulfate is capable of oxidation via oxygen atom transfer to form  $[\text{M}+\text{H}+\text{O}]^+$  as well as hydrogen abstraction to form the unique  $[\text{M}-\text{H}]^+$  species upon ion/ion reactions with doubly protonated peptides. Oxygen atom transfer is capable of proceeding via  $\text{SO}_3$  loss to form  $[\text{M}+2\text{H}+\text{HSO}_5^-]^+$ , which is identical to the complex obtained via ion/ion reactions directly with peroxymonosulfate anions, followed by  $\text{H}_2\text{SO}_4$  loss (Pathway A). Oxygen atom transfer may also proceed via initial loss of  $\text{H}_2\text{SO}_4$  from the complex to form  $[\text{M}+\text{H}+\text{SO}_4]^+$  followed by  $\text{SO}_3$  loss (Pathway B). These processes can result in oxidation at different sites in the peptides, as shown below. Additionally, loss of two sulfuric acid moieties from the complex can occur to form a species of the nominal form  $[\text{M}-\text{H}]^+$  (Pathway C).

These three oxidation pathways are demonstrated in Figure 4 with the peptide ARAMAKA. The ion/ion reaction between doubly protonated ARAMAKA and intact persulfate anions is shown in Figure 4(a). Both proton transfer to form the charge-reduced  $[\text{M}+\text{H}]^+$  species and complex formation occur upon ion/ion reaction. Some fragmentation of the complexes due to energetic transfer conditions from the collision quadrupole to Q3 is also observed, as illustrated by the losses of  $\text{SO}_3$ ,  $\text{H}_2\text{SO}_4$ , and  $\text{H}_2\text{SO}_5$  as well as the formation of the  $[\text{M}+\text{H}+\text{O}]^+$  and  $[\text{M}-\text{H}]^+$  species. Collisional activation of the ion/ion complex,  $[\text{M}+2\text{H}+\text{HS}_2\text{O}_8^-]^+$ , yields product ions from losses of  $\text{SO}_3$ ,  $\text{H}_2\text{SO}_4$ , and  $\text{H}_2\text{SO}_5$ , each of which is discussed below. Additionally, formation of both oxidized species is dominant, though it is unclear what percentage of these species is consecutive from the  $\text{SO}_3$  and/or  $\text{H}_2\text{SO}_4$  losses versus directly from the complex itself. Activation of the product ion from  $\text{SO}_3$  loss exclusively yields the  $[\text{M}+\text{H}+\text{O}]^+$  species via Pathway A for ARAMAKA (Figure 4(c)) though proton transfer may also be observed for some peptides. Activation of the  $\text{H}_2\text{SO}_4$  loss yields both an  $\text{SO}_3$  loss to produce the  $[\text{M}+\text{H}+\text{O}]^+$  species and another  $\text{H}_2\text{SO}_4$  loss to produce the  $[\text{M}-\text{H}]^+$  species (Figure 4(d)). Losses of 30 Da neutral species, corresponding to  $\text{CH}_2\text{O}$  loss, from the complex,  $[\text{M}+\text{H}+\text{O}]^+$ , and  $b_6+\text{O}$  species are also observed. This loss is likely to arise from oxidation of the terminal carbon on the methionine side-chain via a mechanism proposed by Froelich and Reid.<sup>55</sup> Activation of the  $\text{H}_2\text{SO}_5$  loss exclusively undergoes ejection of neutral  $\text{SO}_3$  to form the charge-reduced  $[\text{M}+\text{H}]^+$  species (Supplemental Figure S-5).

While a nominal  $[\text{M}+\text{H}+\text{O}]^+$  species is observed from both the  $\text{SO}_3$  and the  $\text{H}_2\text{SO}_4$  losses, the oxidation does not necessarily occur at the same site(s), as demonstrated in Supplemental Figure S-6 in which the  $[\text{M}+\text{H}+\text{O}]^+$  products from both pathways with ARAMAKA are subjected to CID. Activation of the  $[\text{M}+\text{H}+\text{O}]^+$  species produced via initial  $\text{SO}_3$  loss yields dominant  $\text{CH}_3\text{SOH}$  losses from the oxidized parent and  $b_6$  ions indicating exclusive oxidation of the sulfur atom in the methionine side-chain (Figure S-6(a)). This spectrum is identical to that obtained from activation of the  $[\text{M}+\text{H}+\text{O}]^+$  species produced via activation of the ion/ion complex between doubly protonated ARAMAKA and

peroxymonosulfate anions (see Figure 3(b)). While activation of the  $[M+H+O]^+$  species produced via initial  $H_2SO_4$  loss (Pathway B, Figure S-6(b)) produces many of the same peaks observed in the initial  $SO_3$  loss (Pathway A, Figure S-6(a)) spectrum, there are some key differences. The 64 Da loss corresponding to  $CH_3SOH$  loss from the sulfoxide derivative of the methionine side-chain is observed at a significantly lesser relative abundance for the initial  $H_2SO_4$  loss pathway. This indicates that, while oxygen transfer to the sulfur atom is possible via this mechanism, there are likely other sites oxidized via this pathway as well. Furthermore,  $CH_2O$  loss is observed from initial  $H_2SO_4$  loss (Figure S-6(b)) but not initial  $SO_3$  loss (Figure S-6(a)). As discussed above, this loss indicates that the  $[M+H+O]^+$  ion produced via initial  $H_2SO_4$  loss contains some population oxidized at the terminal carbon on the methionine side-chain. Lastly, small signals corresponding to non-oxidized  $y_4$  and  $y_5$  ions in Figure S-6(b) but not in Figure S-6(a) suggest that some of the oxidation occurs on a site in the peptide other than the methionine residue upon oxidation via Pathway B.

Scheme 3 depicts the proposed mechanisms for oxidation of methionine via ion/ion reactions with intact persulfate anions. Scheme 3(a) illustrates oxidation of the sulfur atom while Scheme 3(b) depicts oxidation of the terminal carbon atom in the methionine side-chain via initial  $H_2SO_4$  loss from an ion/ion complex with intact persulfate. Oxidation of the sulfur atom proceeds via initial proton transfer from the peptide to the reagent followed by nucleophilic attack by the sulfur atom on one of the oxygen atoms in the peroxy bond, resulting in loss of  $H_2SO_4$ . Further rearrangement occurs to eject neutral  $SO_3$  and form a double bond between the sulfur and oxygen atoms (Scheme 3(a)). Oxidation of the terminal carbon atom also undergoes proton transfer from the peptide to the reagent, but is instead followed by abstraction of a hydrogen atom to cleave the peroxy bond, eject neutral  $H_2SO_4$ , and form a bond between the  $\beta$ -peroxy oxygen atom and the terminal carbon atom. The oxidizing oxygen then abstracts a proton, initiating  $SO_3$  loss (Scheme 3(b)). This species can then lose  $CH_2O$  via a mechanism proposed by Froelich and Reid to yield a homocysteine functionality.<sup>55</sup> Both mechanisms illustrated in Scheme 3(a) and (b) proceed via initial  $H_2SO_4$  loss (Pathway B). However, activation of a persulfate-containing ion/ion complex can also generate the  $[M+H+O]^+$  species via initial  $SO_3$  loss (Pathway A) as depicted in Scheme 3(c). Initial  $SO_3$  loss results in a species identical to the complex observed directly from ion/ion reactions between doubly protonated peptides and peroxymonosulfate anions. After  $SO_3$  loss, proton transfer from the peptide to the reagent anion occurs. This is followed by attack by the sulfur atom on the distal oxygen atom with nucleophilic displacement of the peroxy oxygen and simultaneous hydrogen migration as described by Bach, et al., for general reactions between nucleophiles and peroxides.<sup>54</sup> The specific reaction between  $HSO_5^-$  and thioesters has also been previously investigated.<sup>56</sup>

The  $[M-H]^+$  species, which was observed to be generated as a major product only with the persulfate anion, can nominally correspond to the loss of two sulfuric acid moieties from the complex, viz.,  $[M+2H+HS_2O_8^- - 2H_2SO_4]^+$ , or water loss from the oxygen transfer species, viz.,  $[M+H+O-H_2O]^+$ . To investigate which structure should be assigned to this unique species, the CID spectrum of the  $[M-H]^+$  species generated directly from the complex was compared to that of the  $[M+H+O-H_2O]^+$  species (Supplemental Figure S-7). The  $b_6(-2)$  ion is the base peak in both spectra, similar to the spectrum corresponding to CID of the



unmodified peptide in which the  $b_6$  is the base peak (data not shown). Parentheses indicate that the mass of the reported ion is 2 Da less than expected for an unmodified peptide. The 2 Da mass difference can either come from loss of water from an oxygen transfer species or abstraction of two hydrogen atoms. Various b and b(-2) ions are observed throughout both spectra. However, there are 64 Da losses ( $\text{CH}_3\text{SOH}$ ) in the  $[\text{M}+\text{H}+\text{O}-\text{H}_2\text{O}]^+$  product spectrum (Figure S-7(b)) that are not observed in the  $[\text{M}-\text{H}]^+$  product spectrum (Figure S-7(a)). These  $\text{CH}_3\text{SOH}$  losses indicate that there is some population of peptides with oxidized sulfur atoms on the methionine side-chain that have lost water from elsewhere in the peptide. The absence of these losses in the product spectrum of the  $[\text{M}-\text{H}]^+$  ion indicate that no sulfur oxidation is present. Furthermore, small signals due to  $c_2$ ,  $c_3$ , and  $c_5$  ions are observed upon CID of  $[\text{M}-\text{H}]^+$  (Figure S-7(a)) but not  $[\text{M}+\text{H}+\text{O}-\text{H}_2\text{O}]^+$  and a small  $y_5$  ion is observed upon CID for  $[\text{M}+\text{H}+\text{O}-\text{H}_2\text{O}]^+$  (Figure S-7(b)) but not  $[\text{M}-\text{H}]^+$ . These differences suggest that very little, if any, of the  $[\text{M}-\text{H}]^+$  ion population results from water loss from the oxidized species and is likely produced by hydrogen abstraction via loss of two neutral  $\text{H}_2\text{SO}_4$  moieties. It is also worth noting that the abundance of water loss from CID of the  $[\text{M}+\text{H}+\text{O}]^+$  species is low (see Supplemental Figure S-6) compared to the abundance of the  $[\text{M}-\text{H}]^+$  observed from activation of the complex (Figure 4(b)), further supporting the hydrogen abstraction pathway. Oxidation of the non-arginine containing peptide KAKAKAA via ion/ion reaction with intact persulfate anion is shown in Supplemental Figure S-2.

We have found that the formation of the  $[\text{M}-\text{H}]^+$  ion from the  $[\text{M}+2\text{H}]^{2+}$  ion via reaction with the persulfate anion requires the presence of an unprotonated basic site. For instance, doubly protonated GAILGAILR forms an ion/ion complex upon reaction with persulfate anion, but activation of this complex leads predominantly to  $[\text{M}+\text{H}]^+$  (i.e., proton transfer from the peptide to the reagent anion) and, to a lesser degree,  $\text{SO}_3$  loss (Supplemental Figure S-8(a)). Activation of the reaction complex between persulfate and doubly protonated KGAILGAILR, on the other hand, yields the  $[\text{M}-\text{H}]^+$  ion as the base peak along with less abundant formation of the  $[\text{M}+\text{H}+\text{O}]^+$  ion as well as losses of  $\text{SO}_3$ ,  $\text{H}_2\text{SO}_4$ , and  $\text{H}_2\text{SO}_5$  (Figure S-8(b)). Furthermore, triply protonated KGAILGAILR primarily leads to proton transfer and a minor loss of  $\text{SO}_3$  upon activation of the ion/ion complex with persulfate anion (Figure S-8(c)). We also note that species containing easily oxidized residues undergo oxygen transfer in the absence of an unprotonated basic residue to form the  $[\text{M}+\text{H}+\text{O}]^+$  species, as illustrated with the doubly protonated peptide GAGGMGAGGRL in Supplemental Figure S-9, but not the  $[\text{M}-\text{H}]^+$  ion. These trends indicate that the chemistry proceeding via the initial  $\text{H}_2\text{SO}_4$  loss pathway requires an unprotonated basic site to occur. Potential mechanisms for the generation of the  $[\text{M}-\text{H}]^+$  species for each of the three unprotonated basic amino acids are proposed in Scheme 4. The peroxy bond in persulfate is labile (BDE reported to be 33.5 kcal/mol)<sup>57</sup> and is capable of homolytic cleavage to form two radical species. The proposed reaction proceeds via formation of a 6-membered ring upon which radical rearrangement occurs to abstract two hydrogen atoms from adjacent atoms. Additionally, the anionic site on the reagent takes a proton from another basic site along the peptide. This results in ejection of two sulfuric acid moieties and the creation of a double bond between adjacent atoms in the unprotonated amino acid side-chain, or in the case of histidine, between the  $\alpha$ - and  $\beta$ -carbons. The hydrogen atoms abstracted in Scheme 4 were chosen because they had the lowest local bond dissociation energies, as reported by

Benjamin and Julian.<sup>53</sup> While the mechanism proposed in Scheme 4 is one possibility for how the reaction between basic sites and intact persulfate proceeds, there are other possible mechanisms, one of which is presented in Supplemental Scheme S-1.

### **Ion/Ion Reactions with Sulfate Radical Anion ( $\text{SO}_4^{\bullet-}$ ) to Form Peptide Radical Cations ( $[\text{M}]^{\bullet+}$ )**

The creation of gas-phase peptide radical cations is an area of growing interest as the activation of these radical species yields information complementary to that obtained via CID of the protonated species. Strategies to generate radical cations and examination of the fragmentation pathways they undergo have recently been reviewed.<sup>58,33,59</sup> Several strategies for the creation of radical cations via gas-phase ion/ion reactions have also recently been described.<sup>31</sup> Here, we demonstrate the generation of peptide radical cations in the gas-phase via ion/ion reactions with the sulfate radical anion. The reaction proceeds via a long-lived complex of the form  $[\text{M}+2\text{H}+\text{SO}_4^{\bullet-}]^+$ , which undergoes facile loss of sulfuric acid to efficiently yield the peptide radical cation of interest, viz.,  $[\text{M}]^{\bullet+}$ .

The generation of the molecular radical cation from doubly protonated angiotensin III (RVYIHPF) via ion/ion reactions with sulfate radical anion is shown in Figure 5. Activation of the ion/ion complex,  $[\text{M}+2\text{H}+\text{SO}_4^{\bullet-}]^+$ , yields a dominant sulfuric acid loss to produce the peptide radical cation (Figure 5(a)). Further activation of the  $[\text{M}]^{\bullet+}$  yields neutral losses as well as cleavages along the peptide backbone to form the  $a_3$  through  $a_5$ -ions and the  $z_4^{\bullet}$ -ion (Figure 5(b)). The dominant neutral losses observed are  $\text{CO}_2$  from the C-terminus and *p*-quinomethide (106 Da) from the tyrosine side-chain. This spectrum agrees well with the SID spectrum of the angiotensin III radical cation produced by Yang et al. via in-source fragmentation of  $[\text{Co}^{\text{III}}(\text{salen})\text{M}]^+$  and  $[\text{Cu}^{\text{II}}(\text{terpy})\text{M}]^+$  complexes.<sup>60</sup> Activation of the protonated peptide is shown in Figure 5(c) for comparison and is dominated by water and ammonia losses, although *a*-, *b*-, and *y*-type ions are also observed.

Calculations were performed to determine the bond dissociation energy (BDE) of  $\text{H}-\text{SO}_4^-$  and compared to the values previously reported by the Julian group for abstraction of each of the hydrogen atoms on all of the amino acids.<sup>53</sup> The calculated BDE for  $\text{H}-\text{SO}_4^-$  is 454 kJ/mol with an expected error of  $\sim 10$  kJ/mol.<sup>53</sup> This value is higher than most of the values reported for abstracting H-atoms from amino acids with the exception of aromatic carbon radical centers which, in general, are the least stable radical centers. Notably, the  $\text{H}-\text{SO}_4^-$  BDE is higher than those reported for abstraction of hydrogen atoms attached to alpha- (325.5 – 372.3 kJ/mol) and beta- (348.2 – 428.5 kJ/mol) carbons, indicating that the sulfate radical anion can theoretically abstract a hydrogen atom from any peptide upon reaction.<sup>53</sup> Ion/ion reactions with sulfate radical anions are a robust and efficient method of generating peptide radical cations from their protonated dicationic counterparts in the gas-phase.

Peptide cations with multiple radical sites can be generated via sequential ion/ion reactions between multiply protonated peptides and sulfate radical anions. Multiply protonated peptides of the form  $[\text{M}+n\text{H}]^{n+}$  can, in principle, undergo up to  $(n-1)$  additions of sulfate radical anion before neutralization. The  $[\text{M}+5\text{H}]^{5+}$  species of melittin, a large 26-residue peptide, was subjected to ion/ion reactions with sulfate radical anions. Addition of up to three sulfate radical anions was observed, as shown in Figure 6(a). (Addition of a fourth

sulfate radical anion is possible prior to neutralization but is not observed as it is beyond the upper mass-to-charge limit of the instrument.) Activation of the triply adducted species yields sequential losses of sulfuric acid to produce a species of the nominal form  $[M-H]^{2+\dots}$  (Figure 6(b)). Further  $MS^n$  on this peak is dominated by neutral losses with smaller peaks corresponding to both charge-directed (b-, y-type ions) and radical-directed (z- and c-type ions) backbone cleavages (Figure 6(c)). This example also demonstrates the facile ability to generate radical cations from relatively large polypeptide systems via ion/ion reactions with the sulfate radical anion.

## CONCLUSIONS

The generation of various oxidized species, including  $[M-H]^+$ ,  $[M+H+O]^+$ , and  $[M]^{+\bullet}$  peptide cations, from multiply-protonated peptides has been demonstrated using ion/ion reactions with a novel suite of reagents derived from persulfate. Peroxymonosulfate anion,  $HSO_5^-$ , results in oxygen transfer from the reagent to the peptide to yield the  $[M+H+O]^+$  cation upon loss of  $SO_3$ . The intact persulfate anion,  $HS_2O_8^-$ , can transfer oxygen from the reagent to the peptide via two distinct pathways to yield the  $[M+H+O]^+$  cation or it can abstract two hydrogen atoms from unprotonated basic side-chains to yield the  $[M-H]^+$  cation upon ejection of two neutral sulfuric acid molecules. The latter reaction represents a process without precedent in gas-phase ion/ion chemistry. The sulfate radical anion,  $SO_4^{\bullet-}$ , abstracts a hydrogen atom from the peptide to generate the peptide radical cation,  $[M]^{+\bullet}$ , upon loss of one neutral sulfuric acid molecule. These three reagents are either observed directly upon negative nESI of an aqueous solution of persulfate under gentle interface conditions or produced via CID of the reagent under harsher interface conditions thereby providing multiple reagent options within one sample. These results demonstrate that persulfate can provide a unique array of reagent anions for the transformation of multiply protonated peptide ions to other ion-types.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

This work was supported by the National Institutes of Health under Grant GM 45372. Graduate student support for A.L.P. provided by an Emerson Kampen Fellowship.

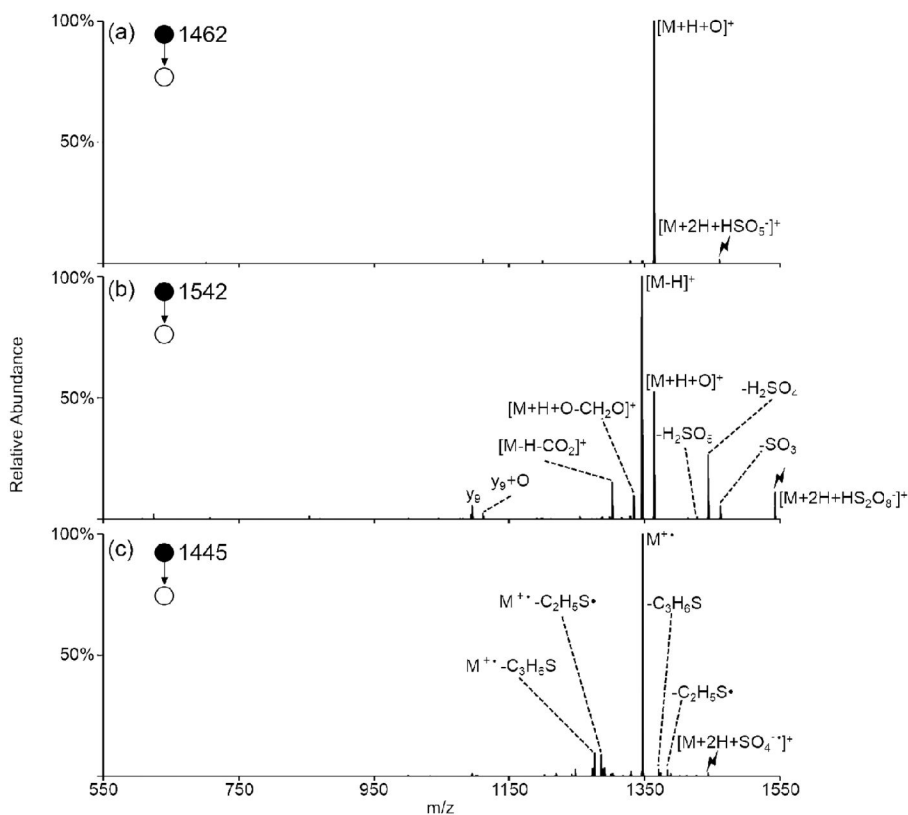
## References

1. McLuckey SA, Mentinova M. Ion/Neutral, Ion/Electron, Ion/Photon, and Ion/Ion Interactions in Tandem Mass Spectrometry: Do we need them all? Are they enough? *J Am Soc Mass Spectrom.* 2011; 22:3–12. [PubMed: 21472539]
2. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. Electrospray Ionization for Mass Spectrometry of Large Biomolecules. *Science.* 1989; 246:64–71. [PubMed: 2675315]
3. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. Electrospray Ionization-Principles and Practice. *Mass Spectrom Rev.* 1990; 9:37–70.
4. Prentice BM, McLuckey SA. Gas-Phase Ion/Ion Reactions of Peptides and Proteins: Acid/Base, Redox, and Covalent Chemistries. *Chem Commun.* 2013; 49:947–965.

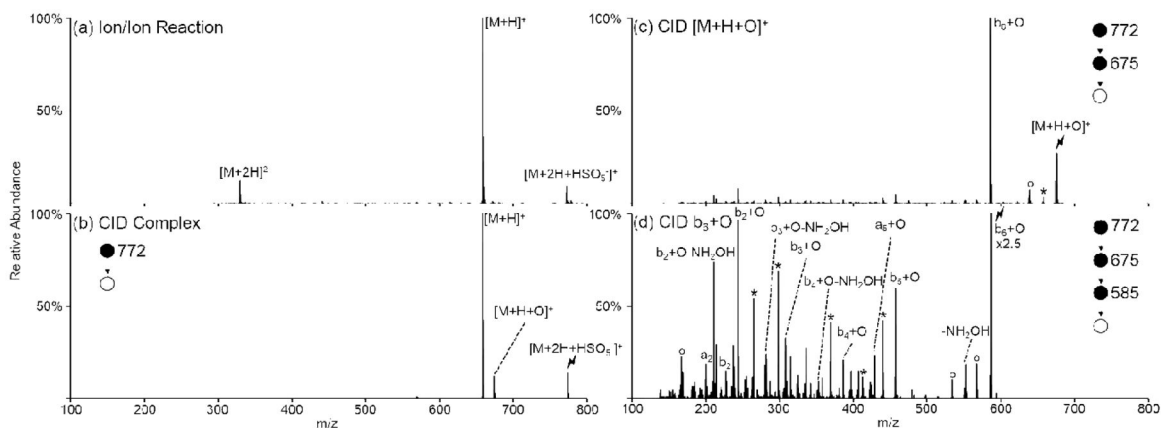
5. McLuckey SA, Stephenson JL Jr. Ion/Ion Chemistry of High-Mass Multiply Charged Ions. *Mass Spectrom Rev.* 1998; 17:369–407. [PubMed: 10360331]
6. Scalf M, Westphall MS, Krause J, Kaufman SL, Smith LM. Controlling Charge States of Large Ions. *Science.* 1999; 283:194–197. [PubMed: 9880246]
7. Good DM, Wirtala M, McAlister GC, Coon JJ. Performance Characteristics of Electron Transfer Dissociation Mass Spectrometry. *Mol Cell Proteomics.* 2007; 6:1942–1951. [PubMed: 17673454]
8. Sohn CH, Chung CK, Yin S, Ramachandran P, Loo JA, Beauchamp JL. Probing the Mechanism of Electron Capture and Electron Transfer Dissociation Using Tags with Variable Electron Affinity. *J Am Chem Soc.* 2009; 131:5444–5459. [PubMed: 19331417]
9. Newton KA, Amunugama R, McLuckey SA. Gas-Phase Ion/Ion Reactions of Multiply Protonated Polypeptides with Metal Containing Anions. *J Phys Chem A.* 2005; 109:3608–3616. [PubMed: 16568152]
10. Payne AH, Glish GL. Gas-phase ion/ion interactions between peptides or proteins and iron ions in a quadrupole ion trap. *Int J Mass Spectrom.* 2001; 204:47–54.
11. Luongo CA, Bu J, Burke NL, Gilbert JD, Prentice BM, Cummings S, Reed CA, McLuckey SA. Selective Removal of Alkali Metal Cations from Multiply-Charged Ions via Gas-Phase Ion/Ion Reactions using Weakly Coordinating Anions. *J Am Soc Mass Spectrom.* 2015; 26:404–415. [PubMed: 25560986]
12. Mentinova M, McLuckey SA. Intra- and Inter-Molecular Cross-Linking of Peptide Ions in the Gas-Phase: Reagents and Conditions. *J Am Soc Mass Spectrom.* 2011; 22:912–921. [PubMed: 21472526]
13. Webb IK, Mentinova M, McGee WM, McLuckey SA. Gas-Phase Intramolecular Protein Crosslinking via Ion/Ion Reactions: Ubiquitin and a Homobifunctional sulfo-NHS Ester. *J Am Soc Mass Spectrom.* 2013; 24:733–743. [PubMed: 23463545]
14. Mentinova M, McLuckey SA. Covalent Modification of Gaseous Peptide Ions with N-Hydroxysuccinimide Ester Reagent Ions. *J Am Chem Soc.* 2010; 132:18248–18257. [PubMed: 21128662]
15. Mentinova M, Barefoot NZ, McLuckey SA. Solution versus Gas-Phase Modification of Peptide Cations with NHS-Ester Reagents. *J Am Soc Mass Spectrom.* 2012; 23:282–289. [PubMed: 22081458]
16. McGee WM, Mentinova M, McLuckey SA. Gas-Phase Conjugation to Arginine Residues in Polypeptide Ions via N-Hydroxysuccinimide Ester-based Reagent Ions. *J Am Chem Soc.* 2012; 134:11412–11414. [PubMed: 22769013]
17. Han H, McLuckey SA. Selective Covalent Bond Formation in Polypeptide Ions via Gas-Phase Ion/Ion Reaction Chemistry. *J Am Chem Soc.* 2009; 131:12884–12885. [PubMed: 19702304]
18. Hassell KM, Stutzman JR, McLuckey SA. Gas Phase Bio-conjugation of Peptides via Ion/Ion Charge Inversion: Schiff Base Formation on the Conversion of Cations to Anions. *Anal Chem.* 2010; 82:1594–1597. [PubMed: 20121142]
19. Stutzman JR, McLuckey SA. Ion/Ion Reactions of MALDI-Derived Peptide Ions: Increased Sequence Coverage via Covalent and Electrostatic Modification upon Charge Inversion. *Anal Chem.* 2012; 84:10679–10685. [PubMed: 23078018]
20. Prentice BM, Gilbert JD, Stutzman JR, Forrest WP, McLuckey SA. Gas-Phase Reactivity of Carboxylic Acid Functional Groups with Carbodiimides. *J Am Soc Mass Spectrom.* 2013; 24:30–37. [PubMed: 23208744]
21. Pilo AL, McLuckey SA. Oxidation of Methionine Residues in Polypeptide Ions Via Gas-Phase Ion/Ion Chemistry. *J Am Soc Mass Spectrom.* 2014; 25:1049–1057. [PubMed: 24671696]
22. Schey KL, Finley EL. Identification of Peptide Oxidation by Tandem Mass Spectrometry. *Acc Chem Res.* 2000; 33:299–306. [PubMed: 10813874]
23. Lagerwerf FM, van de Weert M, Heerma W, Haverkamp J. Oxidation of Oxidized Methionine Peptides. *Rapid Commun Mass Spectrom.* 1996; 10:1905–1910. [PubMed: 9004526]
24. Reid GE, Roberts KD, Kapp EA, Simpson RJ. Statistical and Mechanistic Approaches to Understanding the Gas-Phase Fragmentation Behavior of Methionine Sulfoxide Containing Peptides. *J Proteome Res.* 2004; 3:751–759. [PubMed: 15359728]

25. Qin J, Chait BT. Identification and characterization of posttranslational modifications of proteins by MALDI ion trap mass spectrometry. *Anal Chem.* 1997; 69:3995–4001. [PubMed: 9322436]
26. Jiang XY, Smith JB, Abraham EC. Identification of a MS-MS fragment diagnostic for methionine sulfoxide. *J Mass Spectrom.* 1996; 31:1309–1310.
27. Turek F, Drinkwater DE, McLafferty FW. Gas-Phase Chemistry of CH<sub>3</sub>SOH, CH<sub>2</sub>+SHOH, CH<sub>3</sub>SO•, and •CH<sub>2</sub>SOH by Neutralization-Reionization Mass Spectrometry. *J Am Chem Soc.* 1989; 111:7696.
28. O'Hair RAJ, Reid GE. Neighboring group versus cis-elimination mechanisms for side chain loss from protonated methionine, methionine sulfoxide and their peptides. *Eur J Mass Spectrom.* 1999; 5:325–334.
29. Amunugama M, Roberts KD, Reid GE. Mechanisms for the Selective Gas-phase Fragmentation Reactions of Methionine Side Chain Fixed Charge Sulfonium Ion Containing Peptides. *J Am Soc Mass Spectrom.* 2006; 17:1631–1642. [PubMed: 16935522]
30. Lioe H, Laskin J, Reid GE, O'Hair RAJ. Energetics and Dynamics of the Fragmentation Reactions of Protonated Peptides containing Methionine Sulfoxide or Aspartic Acid via Energy- and Time-Resolved Surface Induced Dissociation. *J Phys Chem A.* 2007; 111:10580–10588. [PubMed: 17914758]
31. Gilbert JD, Fisher CM, Bu J, Prentice BM, Redwine JG, McLuckey SA. Strategies for generating peptide radical cations via ion/ion reactions. *J Mass Spectrom.* 2014; 50:418–426. [PubMed: 25800024]
32. Turek F. Peptide Radicals and Cation Radicals in the Gas Phase. *Chem Rev.* 2013; 113:6691–6733. [PubMed: 23651325]
33. Oh HB, Moon B. Radical-Driven Peptide Backbone Dissociation Tandem Mass Spectrometry. *Mass Spectrom Rev.* 2014; 9999:1–17.
34. Knudsen ER, Julian RR. Fragmentation chemistry observed in hydrogen deficient radical peptides generated from N-nitrosotryptophan residues. *Int J Mass Spectrom.* 2010; 294:83–87.
35. Hao G, Gross SS. Electrospray Tandem Mass Spectrometry Analysis of S- and N-Nitrosopeptides: Facile Loss of NO and Radical-Induced Fragmentation. *J Am Soc Mass Spectrom.* 2006; 17:1725–1730. [PubMed: 16952458]
36. Chu IK, Rodriguez CF, Lau TC, Hopkinson AC, Siu KWM. Molecular radical cations of oligopeptides. *J Phys Chem B.* 2000; 104:3393–3397.
37. Chu IK, Zhao J, Xu M, Siu SO, Hopkinson AC, Siu KWM. Are the radical centers in peptide radical cations mobile? The generation, tautomerism, and dissociation of isomeric  $\alpha$ -carbon-centered triglycine radical cations in the gas phase. *J Am Chem Soc.* 2008; 130:7862–7872. [PubMed: 18512915]
38. Chu IK, Rodriguez CF, Lau TC, Hopkinson AC, Siu KWM. Formation of peptide radical ions through dissociative electron transfer in ternary metal-ligand-peptide complexes. *Eur J Mass Spectrom.* 2011; 17:543–556.
39. Bagheri-Madji E, Ke YY, Orlova G, Chu IK, Hopkinson AC, Siu KWM. Copper-mediated peptide radical ions in the gas-phase. *J Phys Chem B.* 2004; 108:11170–11181.
40. Barlow CK, McFadyen WD, O'Hair RAJ. Formation of cationic peptide radicals by gas-phase redox reactions with trivalent chromium, manganese, iron, and cobalt complexes. *J Am Chem Soc.* 2005; 127:6109–6115. [PubMed: 15839712]
41. Barlow CK, Moran D, Radom L, McFadyen WD, O'Hair RAJ. Metal-mediated formation of gas-phase amino acid radical cations. *J Phys Chem A.* 2006; 110:8304–8315. [PubMed: 16821814]
42. Laskin J, Yang Z, Chu IK. Energetics and dynamics of electron transfer and proton transfer in dissociation of MetalIII(salen)-peptide complexes in the gas phase. 2008; 130:3218–3230.
43. Ly T, Julian RR. Residue-Specific Radical-Directed Dissociation of Whole Proteins in the Gas Phase. *J Am Chem Soc.* 2008; 130:351–358. [PubMed: 18078340]
44. Sun QY, Nelson H, Ly T, Stoltz BM, Julian RR. Side chain chemistry mediates backbone fragmentation in hydrogen deficient peptide radicals. *J Proteome Res.* 2009; 8:958–966. [PubMed: 19113886]

45. Masterson DS, Yin H, Chacon A, Hachey DL, Norris JL, Porter NA. Lysine peroxycarbamates: Free radical-promoted peptide cleavage. *J Am Chem Soc.* 2004; 126:720–721. [PubMed: 14733538]
46. Hodyss R, Cox HA, Beauchamp JL. Bioconjugates for tunable peptide fragmentation: Free radical initiated peptide sequencing (FRIPS). *J Am Chem Soc.* 2005; 127:12436–12437. [PubMed: 16144360]
47. Xia Y, Wu J, Londry FA, Hager JW, McLuckey SA. Mutual Storage Mode Ion/Ion Reactions in Hybrid Linear Ion Trap. *J Am Soc Mass Spectrom.* 2005; 16:71–81. [PubMed: 15653365]
48. Liang W, Xia Y, McLuckey SA. Alternately pulsed nano-electrospray ionization/atmospheric pressure chemical ionization for ion/ion reactions in an electrodynamic ion trap. *Anal Chem.* 2006; 78:3208–3212. [PubMed: 16643016]
49. Londry FA, Hager JW. Mass selective axial ion ejection from a linear quadrupole ion trap. *J Am Chem Soc.* 2003; 14:1130–1147.
50. Frisch, MJ.; Trucks, GW.; Schlegel, HB.; Scuseria, GE.; Robb, MA.; Cheeseman, JR.; Scalmani, G.; Barone, V.; Mennucci, B.; Petersson, GA.; Nakatsuji, H.; Caricato, M.; Li, X.; Hratchian, HP.; Izmaylov, AF.; Bloino, J.; Zheng, G.; Sonnenberg, JL.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Vreven, T.; Montgomery, JA., Jr; Peralta, JE.; Ogliaro, F.; Bearpark, M.; Heyd, JJ.; Brothers, E.; Kudin, KN.; Staroverov, VN.; Kobayashi, R.; Normand, J.; Raghavachari, K.; Rendell, A.; Burant, JC.; Iyengar, SS.; Tomasi, J.; Cossi, M.; Rega, N.; Millam, MJ.; Klene, M.; Knox, JE.; Cross, JB.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, RE.; Yazyev, O.; Austin, AJ.; Cammi, R.; Pomelli, C.; Ochterski, JW.; Martin, RL.; Morokuma, K.; Zakrzewski, VG.; Voth, GA.; Salvador, P.; Dannenberg, JJ.; Dapprich, S.; Daniels, AD.; Farkas, Ö.; Foresman, JB.; Ortiz, JV.; Cioslowski, J.; Fox, DJ. Gaussian 09, Revision D.01. Gaussian, Inc; Wallingford CT: 2009.
51. Hehre WJ, Ditchfield R, Radom L, Pople JA. Molecular orbital theory of the electronic structure of organic compounds. V. Molecular theory of bond separation. *J Am Chem Soc.* 1970; 92:4796–4801.
52. Blanksby SJ, Ellison GB. Bond Dissociation Energies of Organic Molecules. *Acc Chem Res.* 2003; 36:255–263. [PubMed: 12693923]
53. Benjamin NM, Ryan RR. Dissociation energies of X–H bonds in amino acids. *Phys Chem Chem Phys.* 2012; 14:3148–3154. [PubMed: 22286066]
54. Bach RD, Owensby AL, Gonzalez C, Bernhard Schlegel H, McDouall JJW. Nature of the Transition Structure for Oxygen Atom Transfer from a Hydroperoxide. Theoretical Comparison between Water Oxide and Ammonia Oxide. *J Am Chem Soc.* 1991; 113:6001–6011.
55. Froelich JM, Reid GE. Mechanisms for the Proton Mobility-Dependent Gas-Phase Fragmentation Reactions of S-alkyl Cysteine Sulfoxide-Containing Peptide Ions. *J Am Soc Mass Spectrom.* 2007; 18:1690–1705. [PubMed: 17689096]
56. Bunton CA, Foroudian HJ, Kumar A. Sulfide Oxidation and Oxidative Hydrolysis of Thioesters by Peroxymonosulfate Ion. *J Chem Soc Perkin Trans 2.* 1995; 1:33–39.
57. Kolthoff IM, Miller IK. The Chemistry of Persulfate. I. The Kinetics and Mechanism of the Decomposition of the Persulfate Ion in Aqueous Medium. *J Am Chem Soc.* 1951; 73:3055–3059.
58. Turek F. Peptide Radicals and Cation Radicals in the Gas Phase. *Chem Rev.* 2013; 113:6691–6733. [PubMed: 23651325]
59. Hopkinson AC. Radical Cations of Amino Acids and Peptides: Structures and Stabilities. *Mass Spectro Rev.* 2007; 28:655–671.
60. Yang Z, Lam C, Chu IK, Laskin J. The Effect of the Secondary Structure on Dissociation of Peptide Radical Cations: Fragmentation of Angiotensin III and Its Analogues. *J Phys Chem B.* 2008; 112:12468–12478. [PubMed: 18781717]



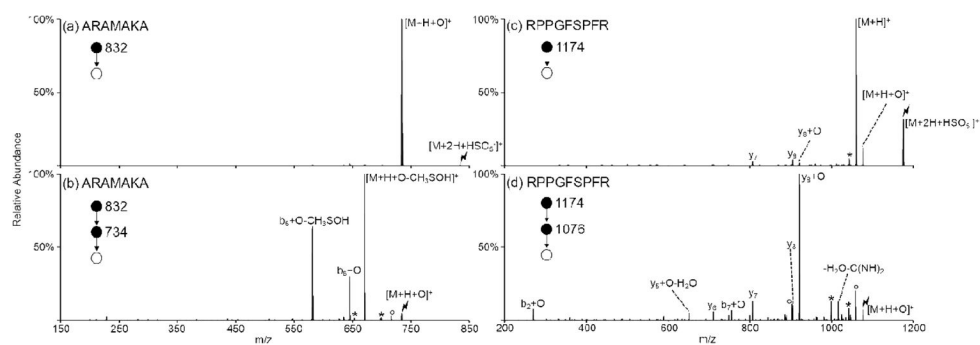
**Figure 1.** Product ion spectra derived from CID of complexes produced via ion/ion reactions between doubly protonated substance P (RPKPQQFFGLM) and (a)  $\text{HSO}_5^-$ , (b)  $\text{HS}_2\text{O}_8^-$ , and (c)  $\text{SO}_4^{\bullet-}$ . The lightning bolt (⚡) indicates species subjected to CID.



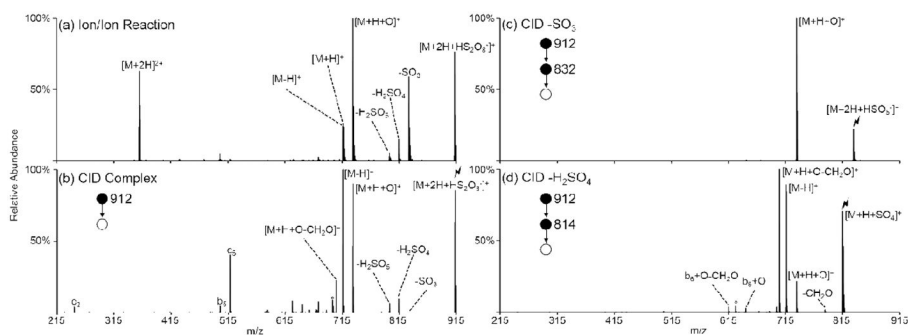
**Figure 2.**

Oxidation of ARAAKA via ion/ion reaction with peroxymonosulfate. (a) Ion/ion reaction between doubly protonated ARAAKA and peroxymonosulfate anion, (b) activation of the ion/ion complex, (c) activation of the  $[M+H+O]^+$  oxidized species, and (d) further activation of the oxidized  $b_6$  ion to further provide sequence information. Asterisks (\*) indicate ammonia losses, degree signs (°) indicate water losses, and the lightning bolt (⚡) indicates species subjected to CID.



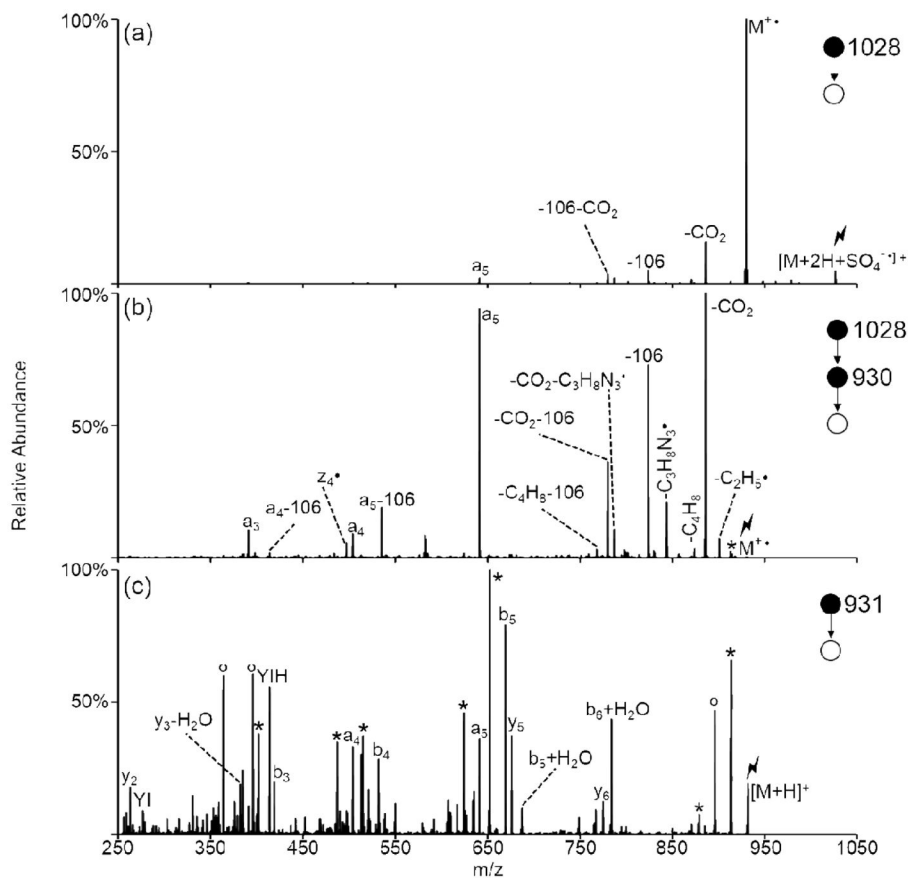


**Figure 3.** Activation of the ion/ion complex produced via the ion/ion reaction between peroxymonosulfate anions and doubly protonated (a) ARAMAKA and (c) bradykinin (RPPGFSPFR). Further CID of the [M+H+O]<sup>+</sup> species produced for (b) ARAMAKA and (d) bradykinin. Asterisks (\*) indicate ammonia losses, degree signs (°) indicate water losses, and the lightning bolt (⚡) indicates species subjected to CID.

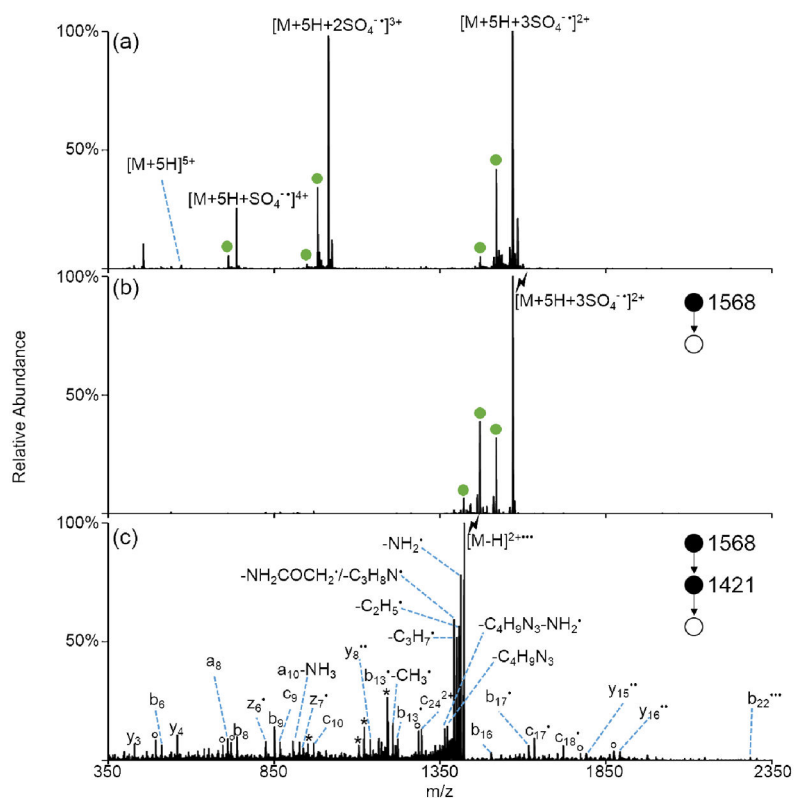


**Figure 4.**

Reaction between doubly protonated ARAMAKA and intact persulfate. (a) Ion/ion reaction between  $[\text{ARAMAKA}+2\text{H}]^{2+}$  and  $\text{HS}_2\text{O}_8^-$ , (b) activation of the ion/ion complex, (c) activation of the  $\text{SO}_3$  loss from the complex, and (d) activation of the  $\text{H}_2\text{SO}_4$  loss from the complex. Asterisks (\*) indicate ammonia losses, degree signs (°) indicate water losses, and the lightning bolt (⚡) indicates species subjected to CID.

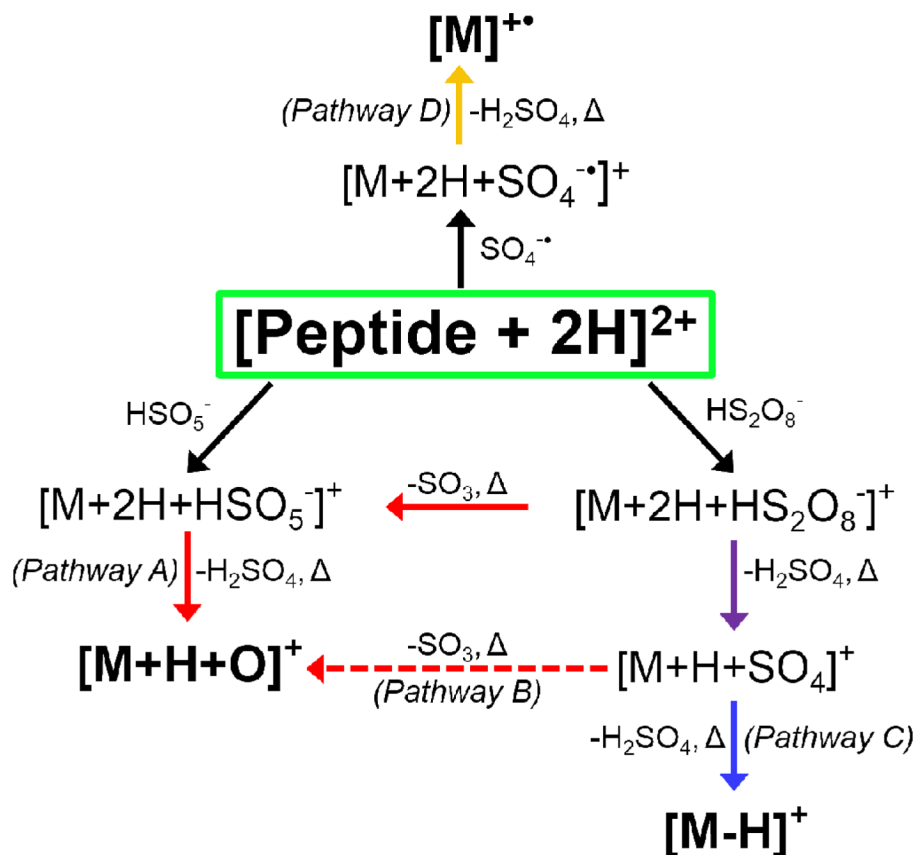


**Figure 5.** Product ion spectra derived from CID of (a) the ion/ion complex between angiotensin III (RVYIHPF) and sulfate radical anion, (b) the radical peptide cation of angiotensin III, and (c) protonated angiotensin III. Asterisks (\*) indicate ammonia losses, degree signs (°) indicate water losses, and the lightning bolt (⚡) indicates species subjected to CID. Loss of 106 Da indicates loss of *p*-quinomethide (C<sub>7</sub>H<sub>6</sub>O) from the tyrosine side-chain.

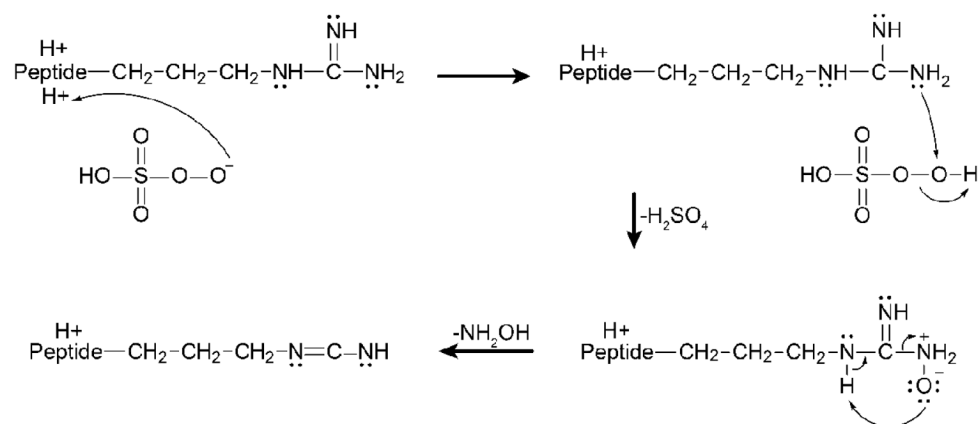


**Figure 6.**

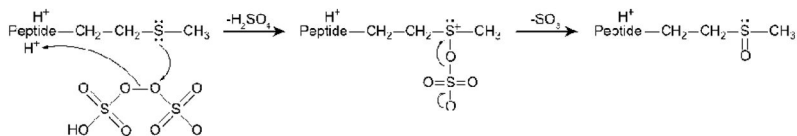
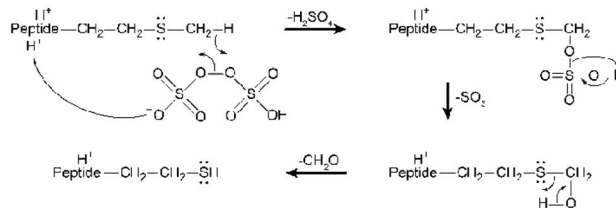
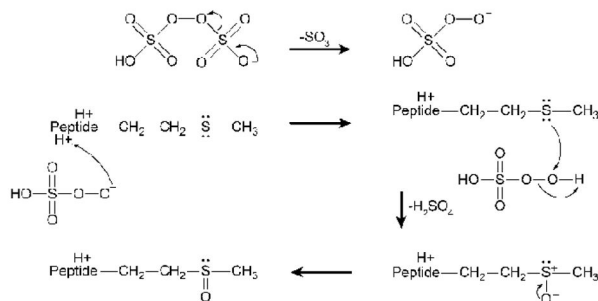
Generation of multiple radical sites in melittin. (a) Ion/ion reaction between sulfate radical anion and melittin  $[M+5H]^{5+}$ . (b) CID of the triply adducted species. (c) CID of the third  $H_2SO_4$  loss from the triply adducted species, viz.,  $[M-H]^{2+***}$ . Asterisks (\*) indicate ammonia losses, degree signs (°) indicate water losses, and the lightning bolt (⚡) indicates species subjected to CID. Green circles indicate  $H_2SO_4$  loss.

**Scheme 1.**

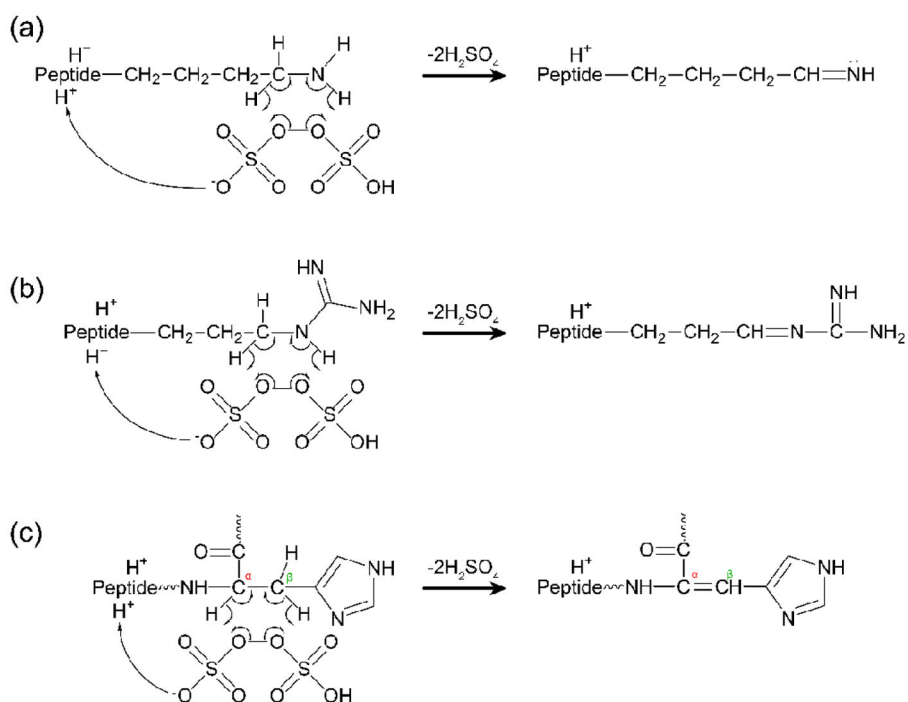
Summary of oxidative pathways available to a doubly protonated peptide upon ion/ion reaction with each of the reagents derived from persulfate and subsequent  $MS^n$  (CID indicated by  $\Delta$ ) of the complexes. Black arrows indicate an ion/ion reaction, the orange arrow indicates the pathway to create a radical peptide cation, the blue arrow indicates the pathway to form the hydrogen-deficient  $[M-H]^+$  species, red arrows indicate oxygen transfer pathways to form the  $[M+H+O]^+$  species with the dashed arrow indicating a pathway that results in a structure different than that obtained via solid-arrow pathways, and the purple arrow indicates the generation of a species capable of proceeding via either the oxygen transfer or the hydrogen abstraction pathway.

**Scheme 2.**

Mechanism of  $[M+H+O]^+$  formation via ion/ion reaction between peroxymonosulfate anion and an arginine-containing peptide and  $\text{NH}_2\text{OH}$  loss from oxidized arginine side-chain.

(a) Initial loss of  $\text{H}_2\text{SO}_4$ , Oxidation of sulfur atom(b) Initial loss of  $\text{H}_2\text{SO}_4$ , Oxidation of terminal carbon atom(c) Initial loss of  $\text{SO}_3$ , Oxidation of sulfur atom**Scheme 3.**

Mechanism of reaction for methionine residues and  $\text{HS}_2\text{O}_8^-$  to yield oxidation of (a) the sulfur atom, and (b) the carbon atom, via initial  $\text{H}_2\text{SO}_4$  loss and (c) oxidation of the sulfur atom via initial  $\text{SO}_3$  loss.

**Scheme 4.**

Mechanisms to form  $[M-H]^+$  species upon reaction between intact persulfate anion and (a) lysine, (b) arginine, and (c) histidine.