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Gas-Phase Oxidation of Neutral Basic Residues in Polypeptide Cations by Periodate

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Abstract

The gas-phase oxidation of doubly protonated peptides containing neutral basic residues to various products, including $[M+H+O]^+$, $[M-H]^+$, and $[M-H-NH_3]^+$, is demonstrated here via ion/ion reactions with periodate. It was previously demonstrated that periodate anions are capable of oxidizing disulfide bonds and methionine, tryptophan, and S-alkyl cysteine residues. However, in the absence of these easily oxidized sites, we show here that systems containing neutral basic residues can undergo oxidation. Furthermore, we show that these neutral basic residues primarily undergo different types of oxidation (e.g., hydrogen abstraction) reactions than those observed previously (i.e., oxygen transfer to yield the $[M+H+O]^+$ species) upon gas-phase ion/ion reactions with periodate anions. This chemistry is illustrated with a variety of systems including a series of model peptides, a cell-penetrating peptide containing a large number of unprotonated basic sites, and ubiquitin, a roughly 8.6 kDa protein.

Graphical Abstract

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Keywords

Ion/ion reaction; oxidation; periodate reagent; tandem mass spectrometry

INTRODUCTION

The manipulation of multiply charged analyte ions generated by electrospray ionization (ESI) via ion/ion reactions has been demonstrated on a variety of instrument platforms [1]. Ion/ion reactions occur either via long-range charge transfer of small charged particles (i.e., through a ‘hopping’ mechanism) or via the formation of a long-lived complex [2]. The most commonly employed ion/ion interactions are electron and proton transfer reactions. Electron transfer dissociation (ETD) [3], and its negative mode counterpart, negative ETD (NETD) [4,5], via ion/ion reactions have been shown to be particularly useful in the sequence analysis of peptides and proteins as they yield complementary ions to those obtained upon CID and retain labile post-translational modifications [6,7]. Proton transfer charge-reduction ion/ion reactions have been used to simplify mass spectra by spreading overlapping peaks over a larger m/z range, thus effectively increasing peak capacity [8,9,10]. The transfer of multiple protons has been used to charge invert (viz., change the polarity of) analytes and in doing so reduce chemical noise in complex mixtures [11], increase structural information gained from lipids upon collision-induced dissociation (CID) [12], and differentiate phosphatidylcholine and phosphatidylethanolamine isomers [13].

Several covalent modifications of bioanalytes have been demonstrated via ion/ion reactions [1]. Recent examples of these modifications include the esterification of carboxylate groups via cation transfer [14], gas-phase conjugation of alkynes and azides via ‘Click’ chemistry reactions [15], the N- and C-terminal extension of peptides via conjugation with N-hydroxysuccinimide esters [16] and Woodward’s Reagent K [17], respectively, and the conjugation of aldehydes and amines via gas-phase Schiff base chemistry [18,19,20,21,22]. Gas-phase ion/ion reactions have several benefits over traditional solution-phase derivatizations including much shorter reaction times (<1 sec), reduction of side reactions via mass isolation of reactants, and control over the extent of reaction (viz., amount of product formed and number of modifications that occur) [1]. Furthermore, some unique chemistries can be observed upon gas-phase ion/ion reactions that are difficult to observe or isolate in the solution phase [1,23].

The gas-phase oxidation of disulfide bonds [23], S-alkyl cysteine residues [24], and methionine and tryptophan residues [25] via ion/ion reactions with periodate anion has recently been demonstrated. Furthermore, the oxidation of protonated polypeptides to $[M+H+O]^+$, $[M-H]^+$, and $M^{+\bullet}$ species upon ion/ion reactions with a suite of reagents derived from persulfate has been demonstrated [26]. These oxidation ion/ion reactions have been used to determine which cysteine residues are involved in disulfide bonds, identify and localize certain post-translational modifications (e.g., prenylation), and obtain additional primary structural information. Based on the initial studies it appeared that, in unmodified non-disulfide linked systems, methionine and tryptophan were the only amino acids that could undergo oxidation upon ion/ion reaction with periodate anion [25]. Here, we show that

peptides containing neutral basic sites can also undergo oxidation upon ion/ion reactions with periodate anion in the absence of the easily oxidized residues (viz., Met and Trp). Multiple oxidized species, including the $[M+H+O]^+$, $[M-H]^+$, and $[M-H-NH_3]^+$ species, are observed upon activation of the peptide-periodate ion/ion complex. Potential mechanisms to generate each species are discussed. Finally, we demonstrate the oxidation of various cell-penetrating peptides and a protein, systems which typically contain a high density of unprotonated basic residues, with periodate anion upon ion/ion reaction.

EXPERIMENTAL SECTION

Materials

Methanol and glacial acetic acid were purchased from Mallinckrodt (Phillipsburg, NJ). KAKAKAA was synthesized by NeoBioSci (Cambridge, MA), RARARAA was synthesized by CHI Scientific (Maynard, MA), HAHAAHA was synthesized by Pepnome Ltd. (Shenzhen, China), and RGAGGRGAGGHL was synthesized by CPC Scientific (Dublin, CA). The cell-penetrating MAP peptide was obtained from AnaSpec (Fremont, CA). Ubiquitin and sodium periodate were purchased from Sigma Aldrich (St. Louis, MO). All peptide stock solutions for positive nanoelectrospray were prepared in a 49.5/49.5/1 (v/v/v) solution of methanol/water/acetic acid at an initial concentration of ~1 mg/mL and diluted 100-fold prior to use. The periodate solution was prepared in a 50/50 (v/v) solution of methanol/water at a concentration of ~1 mg/mL and diluted 10-fold prior to use.

Mass Spectrometry

All peptide 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 [27]. Multiply protonated peptides and singly charged anion reagent populations were sequentially injected into the instrument via alternately pulsed nano-electrospray (nESI), independently isolated in the Q1-mass filter, and injected into the q2 reaction cell where they were allowed to react for 20 – 1000 ms [28]. The ion/ion reaction products were then transferred to Q3, where the complex was subjected to further characterization via MS^n and mass analysis using mass-selective axial ejection (MSAE) [29].

The ubiquitin experiment was performed on a TripleTOF 5600 mass spectrometer (AB Sciex, Concord, ON, Canada), previously modified for ion/ion reactions. The multiply protonated ubiquitin cations were ionized via positive nESI, isolated in transit through Q1 and stored in q2. The reagent anions (periodate for oxidation and perfluoro-1-octanol dimer anions for proton transfer) were then ionized via negative nESI, isolated in transit through Q1 and mutually stored with the protein cations in q2 for 10 ms. The ion/ion reaction products were then subjected to dipolar direct current (DDC) CID, a broadband heating technique where the ions are moved from the center of the trap towards one of the rods to induce rf heating [30], to remove the adducts and generate the oxidized products. Product ions were then transferred to the TOF for mass analysis.

RESULTS AND DISCUSSION

As demonstrated previously, peptides containing easily oxidized moieties (i.e., methionine residues [25], S-alkylated cysteine residues [24], and disulfide bonds [23]) yield dominant $[M+H+O]^+$ species due to oxygen transfer from the reagent anion to the reactive side-chain upon activation of peptide/periodate ion/ion complexes. Here, we show that peptides lacking easily oxidized residues can also undergo oxidation upon ion/ion reaction with IO_4^- . Doubly protonated peptides lacking easily oxidized functional groups were subjected to ion/ion reactions with IO_4^- . Upon reaction the doubly protonated peptide either transfers a proton to the periodate anion to yield the charge-reduced species or generates a long-lived ion/ion complex, viz., $[M+2H+IO_4]^+$ with the reagent anion. Activation of this long-lived complex either results in proton transfer from the peptide dication to the reagent anion to generate the charge-reduced $[M+H]^+$ species via loss of HIO_4 or undergoes covalent oxidation chemistry. The covalent chemistry can occur through multiple pathways: (i) oxygen transfer from the reagent anion to the peptide cation to generate the $[M+H+O]^+$ species as well as HIO_3 , (ii) abstraction of two hydrogen atoms from the peptide by the reagent anion to generate the $[M-H]^+$ species along with H_3IO_4 (or HIO_3+H_2O , though these are not observed as sequential losses), or (iii) abstraction of two hydrogen atoms and an ammonia group to generate the $[M-H-NH_3]^+$ species via loss of $I(OH)_4NH_2$ (or $HIO_3+H_2O+NH_3$). These pathways are summarized in Scheme 1.

Oxidation of Neutral Arginine Residues

To demonstrate this chemistry, a series of doubly protonated model peptides of the form XAXAXAA (X is Arg, Lys, or His) was subjected to ion/ion reactions with IO_4^- . The ion/ion reaction between doubly protonated RARARAA and IO_4^- is shown in Figure 1. Dominant complex formation to generate the $[M+2H+IO_4]^+$ is observed, along with minor formation of the $[M+H]^+$ species due to proton transfer from the peptide dication to the reagent anion. Results from the activation of the ion/ion complex are shown in Figure 2(a) and show dominant loss of H_3IO_4 to generate the $[M-H]^+$ peak. The $[M+H]^+$ and $[M-H-NH_3]^+$ species are observed at moderate to high abundances. Minor formation of the $[M+H+O]^+$ species via loss of HIO_3 is also observed. The inset in Figure 2(a) shows a zoomed-in region around the $[M+H]^+$ and $[M-H]^+$ peaks to show the relative contributions of each. While the $[M-H]^+$ species could nominally correspond to a water loss from the $[M+H+O]^+$ species, this is likely not the case since (i) the $[M+H+O]^+$ species is at such a low abundance, and (ii) CID of the $[M+H+O]^+$ species results in only minor water loss (Figure 2(b)). While there was not enough signal to do MS^n on the water loss from the $[M+H+O]^+$ species for this system, experiments with other peptides yielded different spectra upon activation of the $[M-H]^+$ species formed directly from the complex versus upon activation of the $[M+H+O-H_2O]^+$ species, indicating that they are indeed different structures (*vide infra*).

Results from the activation of the $[M+H+O]^+$ species are shown in Figure 2(b) and primarily show loss of $C(NH)_2$ (42 Da) from a neutral arginine side-chain. An additional step of activation of the $[M+H+O-C(NH)_2]^+$ species was done to obtain additional information about the location of the modification (Supplemental Figure S-1). There are several modified and unmodified b- and y-ions indicating multiple sites of oxidation. Activation of the $[M-$

H]⁺ species is shown in Figure 2(c) and results in a dominant ammonia loss as well as several smaller b- and y-type fragments. The red triangles in Figure 2(c) indicate fragments that are shifted 2 Da lower than the expected mass, i.e., they are [b/y-H]⁺ type ions. The y_5/y_6^{Δ} ions indicate that at least two different structures corresponding to oxidation of different sites are present and the presence of only modified b_2^{Δ} and b_6^{Δ} ions support the conclusion that the modification is occurring on arginine side-chains. Activation of the [M-H-NH₃]⁺ species generated upon dissociation of the ion/ion complex is shown in Figure 2(d). Blue diamonds indicate fragments that are shifted 19 Da lower than expected for unmodified fragments, i.e., they are [b/y-H-NH₃]⁺ fragment ions. The [M-H-NH₃]⁺ species could nominally correspond to loss of I(OH)₄NH₂ (or HIO₃+H₂O+NH₃) directly from the complex or sequential loss of ammonia from the [M-H]⁺ species. For arginine-containing systems, it appears that this peak corresponds to sequential loss of ammonia from the [M-H]⁺ species as CID of the ammonia loss from the [M-H]⁺ peak (see Supplemental Figure S-2) is almost identical to that obtained for CID of the [M-H-NH₃]⁺ peak generated directly from CID of the ion/ion complex (Figure 2(d)). However, this is not the case for lysine-containing systems (*vide infra*).

Oxidation of Neutral Lysine Residues

Doubly protonated KAKAKAA was subjected to ion/ion reactions with IO₄⁻. Results of the activation of the ion/ion complex are shown in Figure 3(a). While proton transfer from the peptide dication to the reagent anion to generate the [M+H]⁺ species is the dominant pathway observed for this system, there is abundant formation of the [M-H]⁺ and [M-H-NH₃]⁺ species. Once again, the oxygen transfer pathway to generate the [M+H+O]⁺ species is minor in abundance. Activation of the [M+H+O]⁺ species is shown in Figure 3(b) and results in a wide variety of oxidized and unmodified b- and y-ions. These pairs of modified and unmodified ions (y₅/y₅+O, y₆/y₆+O, b₂/b₂+O, b₃/b₃+O, b₄/b₄+O) indicate multiple oxidation sites. For this system, the [M-H]⁺ species is lower in abundance relative to the arginine-containing system discussed above. To ensure that the [M-H]⁺ ion is not simply derived from a water loss from the [M+H+O]⁺ species, the spectrum resulting from CID of the [M-H]⁺ ion generated directly from the complex (Figure 3(c)) was compared to that from CID of the water loss from the [M+H+O]⁺ ion (Supplemental Figure S-3). These spectra are indeed quite different, confirming that the [M-H]⁺ peak observed is not simply derived from a sequential water loss from the [M+H+O]⁺ species. Also of note in Figure 3(c) is the lack of ammonia loss from the [M-H]⁺ species. This indicates that, unlike with RARARAA, the [M-H-NH₃]⁺ peak is derived from a I(OH)₄NH₂ loss directly from the complex as it cannot be a sequential ammonia loss from the [M-H]⁺ species. The spectrum derived from activation of the [M-H-NH₃]⁺ species is shown in Figure 3(d) and predominantly results in ions that are shifted in mass by 19 Da, viz., [b/y-H-NH₃]⁺ ions. Some smaller unmodified ions are also present, once again indicating that the modification can occur on any of the lysine side-chains.

Comparison of Charged and Neutral Basic Side-chains

Triply charged KAKAKAA and RARARAA were each subjected to ion/ion reactions with IO₄⁻. Triply charged KAKAKAA exclusively transferred a proton to the periodate reagent anion to generate the charge-reduced species, no doubly charged complex, viz., [M+3H+IO₄]²⁺ was observed (Supplemental Figure S-4). Single and double proton transfer to

generate the $[M+2H]^{2+}$ and $[M+H]^+$ species, respectively, were observed, along with proton transfer followed by complex formation to generate the singly charged, singly adducted complex, viz., $[M+2H+IO_4]^+$. The latter complex is nominally the same as the one described in the previous section derived from the ion/ion reaction between doubly protonated KAKAKAA and periodate anion and both gave essentially the same product ion spectrum upon CID (see Figure 3a). In this case, oxidation would be expected because one of the protonation sites was converted to a neutral site in the first ion/ion reaction. The lack of a singly adducted doubly charged complex, viz., $[M+3H+IO_4]^{2+}$, may be due to relatively weak electrostatic interactions between periodate and protonated primary amines and the somewhat higher ion/ion reaction exothermicity associated with the reaction of a triply charged cation with periodate compared to a doubly charged version. The ion/ion reaction between triply protonated RARARAA and IO_4^- is shown in Figure 4 and results in generation of a doubly charged complex, viz., $[M+3H+IO_4]^{2+}$, singly charged monoadducted complex, viz., $[M+2H+IO_4]^+$, and singly charged doubly adducted complex, viz., $[M+3H+2IO_4]^+$, along with a minor extent of proton transfer to generate the $[M+2H]^{2+}$ species. Protonated arginine side-chains engage in stronger electrostatic interactions with anions than do protonated lysine side-chains [31], likely contributing to the observation of more abundant ion/ion complexes. Activation of the doubly adducted complex, viz., $[M+3H+2IO_4]^+$ is shown in Figure 4(b) and results in loss of HIO_4 to generate the $[M+2H+IO_4]^+$ prior to sequential losses that generate the same oxidized species as observed in Figure 2. Further isolation and activation of the $[M+2H+IO_4]^+$ species in Figure 4(b) generates a spectrum identical to that shown in Figure 2(a) for CID of the same complex generated via one addition of periodate to doubly protonated RARARAA (data not shown). Activation of the $[M+3H+IO_4]^{2+}$ species results almost exclusively in proton transfer, though there is a small amount of oxygen transfer species observed (Figure 4(c)). Activation of the $[M+2H+O]^{2+}$ (Figure 4(d)) species leads to a spectrum similar to that derived from activation of the $[M+H+O]^+$ species (Figure 2(b)) as neutral losses of ammonia and $C(NH)_2$ are the dominant products observed. The lack of doubly oxidized species and the extremely minor formation of singly oxidized species for the $[M+3H+IO_4]^{2+}$ species indicate that the oxidation chemistry described here likely requires the presence of neutral basic residue.

Oxidation of Neutral Histidine Residues

Doubly protonated HAHAAHAA was also subjected to ion/ion reactions with IO_4^- . Unlike the other two systems described above, activation of the ion/ion complex resulted exclusively in proton transfer, no oxidation chemistry was observed (Supplemental Figure S-5). This lack of oxidation may have been due to the relatively weak electrostatic interaction between protonated histidine and periodate compared the analogous interactions in RARARAA and KAKAKAA. If the barrier to proton transfer is lower than any barriers associated with oxidation reaction channels, proton transfer will dominate upon activation of the complex. To determine if histidine is capable of oxidation chemistry under any likely scenarios, doubly protonated RGAGGRGAGGHL was subjected to ion/ion reactions with IO_4^- (Supplemental Figure S-6). Since arginine has a much higher proton affinity than histidine, it is likely that both of the arginine residues are protonated in this system, and as such, should not react. Furthermore, the electrostatic interaction between protonated arginine and periodate is expected to be stronger than that for protonated histidine and periodate.

Activation of the ion/ion complex resulted predominantly in proton transfer. However, a minor peak corresponding to the $[M+H+O]^+$ species was observed (Supplemental Figure S-6(a)). Neither the $[M-H]^+$ nor the $[M-H-NH_3]^+$ species were observed. Activation of the $[M+H+O]^+$ species (Supplemental Figure S-6(b)) resulted in a variety of modified and unmodified ions indicating a mixture of structures. The non-oxidized b_6 ion indicates that at least some population of ions is oxidized at the histidine residue (potential mechanism shown in Supplemental Scheme S-1) as both of the arginine residues are contained within this fragment. Overall, however, oxidation of histidine residues upon ion/ion reaction with IO_4^- is an extremely minor pathway.

Proposed Mechanisms for the Oxidation of Neutral Basic Sites

This work was chiefly devoted to addressing the possibility for oxidation chemistry associated with unprotonated arginine, lysine, and histidine residues, and is not a detailed mechanistic study. Nevertheless, possible mechanisms for the oxidation of neutral arginine and lysine side-chains to the $[M-H]^+$ species are provided in Scheme 2. Similar to the mechanisms proposed for the generation of the same species via ion/ion reactions with persulfate anion [26], the reaction proceeds via abstraction of two hydrogen atoms from adjacent carbon and nitrogen atoms with concurrent proton transfer from the peptide cation to the reagent anion. The hydrogen atoms abstracted in Scheme 2 were chosen because they had the lowest reported local bond dissociation energies [32]. This results in a degree of unsaturation along the neutral lysine or arginine side-chain upon ejection of H_3IO_4 . It is unclear whether H_3IO_4 fragments into the more stable HIO_3+H_2O pair as these are not observed as sequential losses. While persulfate and periodate are both capable of generating various oxidized species, persulfate anion is a stronger oxidizing reagent anion as the ratio of oxidation to proton transfer is typically higher for persulfate than for periodate and persulfate oxidizes peptides both containing and lacking easily oxidized residues (e.g., Met, Trp) to multiple forms (periodate exclusively oxidizes these peptides to $[M+H+O]^+$).

Potential mechanisms for the oxidation of neutral lysine side-chains to yield $[M-H-NH_3]^+$ species are proposed in Scheme 3. The first mechanism (Scheme 3(a)) is proposed for N-terminal lysine residues and occurs via nucleophilic attack on the periodate by the N-terminus and the primary amine on the lysine side-chain. This forms an eight-membered ring which then rearranges to form a more stable six-membered ring. The periodate then abstracts a hydrogen atom from the alpha-carbon of the lysine residue and undergoes rearrangement to eject $I(OH)_4NH_2$ and generate the structure shown in Scheme 3(a). It is unclear whether the $I(OH)_4NH_2$ fragments into its more stable components, viz., $HIO_3+H_2O+NH_3$. The second mechanism proposed in Scheme 3(b) is for the oxidation of an internal lysine residue and differs from Scheme 3(a) in that the backbone nitrogen is now an amide instead of the N-terminus. Since the amide nitrogen only has one hydrogen atom, the eight-to-six-membered ring rearrangement requires the abstraction of a proton from elsewhere on the peptide and the final product now has a fixed charge. Mechanisms to generate the $[M+H+O]^+$ species from neutral arginine and lysine side-chains are proposed in Supplemental Scheme S-2. Theoretical calculations or isotopic labeling experiments could potentially be done to further support the proposed mechanisms.

Oxidation of a Cell-Penetrating Peptide

Electrospray ionization of peptides in the positive mode often leads to the protonation of all basic residues, which renders them unreactive with respect to oxidation if they do not contain one of the readily oxidized moieties (e.g., Met, Trp, alkylated Cys, or disulfide bond). Cell-penetrating peptides, a small but important class of peptides, often have a large density of basic residues as the positive charge helps in the association of the peptide with the negatively charged proteoglycans and phospholipids on the cell surface [33,34,35,36,37]. In these peptides, a large fraction of the positive ions can have unprotonated basic sites. Doubly protonated model amphipathic peptide (MAP), a cell-penetrating peptide of the sequence KLALKLALKALKLA that can be conjugated with large molecules and used as a drug-delivery vehicle [38,39], was subjected to ion/ion reactions with IO_4^- . Similar to KAKAKAA, activation of the ion/ion complex resulted in dominant formation of the proton transfer $[\text{M}+\text{H}]^+$ species along with generation of peaks corresponding to $[\text{M}-\text{H}]^+$ and $[\text{M}-\text{H}-\text{NH}_3]^+$ at moderate to high abundance (Figure 5(a)). The oxygen transfer species is small but has sufficient signal for an additional step of CID, as shown in Figure 5(b). A large variety of modified and unmodified b-ions are observed upon activation of the $[\text{M}+\text{H}+\text{O}]^+$ species, several of which indicate multiple oxidation sites within the peptide. Similarly, the $[\text{M}-\text{H}]^+$ and $[\text{M}-\text{H}-\text{NH}_3]^+$ species were subjected to CID and the resulting spectra are shown in Figure 5(c) and (d), respectively. The majority of peaks in these spectra are modified b-ions, though at least some pairs of modified and unmodified ions are present in both spectra that indicate oxidation at multiple sites. Overall, the CID spectra of each of these oxidized species is similar to the control spectrum taken for CID of the protonated peptide (Supplemental Figure S-7). While the fragmentation pattern does not change significantly, these oxidized species could be useful in the identification of species containing a large number of basic residues (and lacking easily oxidized residues, e.g., Met, Trp) as only peptides containing unprotonated basic sites are susceptible to this type of oxidation chemistry.

Oxidation of Ubiquitin

The oxidation of protein ions upon ion/ion reactions with periodate anion was also investigated. Proteins are extremely important from a biological standpoint and often contain a large number of unprotonated basic sites upon positive nESI, making them a good example of a system in which this oxidation chemistry could be dominant. Ubiquitin is an ~8.6 kDa protein with 13 basic sites, 11 of which are arginine or lysine residues. The $[\text{M}+7\text{H}]^{7+}$ species of ubiquitin, which contains 6 unprotonated basic sites, was subjected to ion/ion reactions with IO_4^- . Since ubiquitin also has one methionine residue that can also undergo oxidation, two periodate anions were adducted to the protein to generate the $[\text{M}+7\text{H}+2\text{IO}_4]^{5+}$ species to increase the likelihood of neutral basic site oxidation. Dipolar direct current (DDC) CID, a broadband heating technique, was used to remove the periodate adducts and generate the oxidized species (Figure 6(a)). In addition to the generation of the $[\text{M}+5\text{H}]^{5+}$ species via two proton transfer reactions, there is evidence for the generation of a variety of oxidized products. This includes ions consistent with two oxygen transfer events ($[\text{M}+5\text{H}+2(\text{O})]^{5+}$), one oxygen transfer and one proton transfer event ($[\text{M}+5\text{H}+\text{O}]^{5+}$), one oxygen transfer and one hydrogen abstraction event ($[\text{M}+3\text{H}+\text{O}]^{5+}$), one proton transfer and one hydrogen abstraction event ($[\text{M}+3\text{H}]^{5+}$), two hydrogen abstraction events ($[\text{M}+\text{H}]^{5+}$),

one proton transfer and one ammonia/hydrogen abstraction event ($[M+3H-NH_3]^{5+}$), one hydrogen abstraction and one ammonia/hydrogen abstraction event ($[M+H-NH_3]^{5+}$), and two ammonia/hydrogen abstraction events ($[M+H-NH_3]^{5+}$). There is a minor signal consistent with $[M+5H+3(O)]^{5+}$, which likely arises from a small population of ubiquitin oxidized in solution and present in the isolation of the precursor ion population (see below). This solution-oxidized peak can also contribute to a minor degree to some of the other distributions observed in Figure 6(a) as well. The same experiment using the perfluoro-1-octanol dimer anion, a proton transfer reagent, instead of periodate resulted in the product ions shown in Figure 6(b). Note the small signal arising from the solution oxidized protein present in the precursor ion population. Comparison of Figure 6(a) and (b) illustrates a shift to lower mass upon oxidation ion/ion reactions due to the oxidation pathways available to unprotonated basic sites. Ramped CID, where the amplitude of the rf is scanned to bring a range of m/z values into resonance with the auxiliary waveform used for ion trap CID, over this entire range of oxidized species was done and compared to the control, i.e., ramped CID over the same mass range for the $[M+5H]^{5+}$ species resulting from charge-reduction ion/ion reactions with PFO (Supplemental Figure S-8). The resulting spectra illustrate very different fragmentation patterns, which we are currently studying in more detail.

CONCLUSIONS

In this work, we have demonstrated the oxidation of neutral basic sites in polypeptides and a protein via ion/ion reactions with periodate anions. Ion/ion reactions between periodate anions and neutral arginine-containing cations can result in generation of $[M+H+O]^+$ species via oxygen transfer from periodate to the peptide cation in a minor process or $[M-H]^+$ species via abstraction of two hydrogen atoms from the arginine side-chain by the reagent anion as the major oxidation process. Ion/ion reactions between periodate anions and neutral lysine-containing cations can result in generation of both of these two products, viz., $[M+H+O]^+$ and $[M-H]^+$, and an additional species, $[M-H-NH_3]^+$. While the latter ion-type is also observed for neutral arginine-containing peptides, it is generated via a sequential ammonia loss from the $[M-H]^+$ peak instead of directly from the complex as is the case for neutral lysine. Histidine was also determined to undergo oxygen transfer chemistry upon ion/ion reaction with IO_4^- , though to a significantly lesser extent. This oxidation chemistry was further demonstrated with a cell-penetrating peptide containing a high density of lysine residues and ubiquitin, an ~8.6 kDa protein. It is important to recognize the possibility for such reactions when using periodate as a reagent anion for the structural characterization of polypeptide ions. However, in the cases of methionine, alkylated cysteines, and disulfide linkages, oxygen transfer is the clearly dominant channel whereas it is observed to be only a very minor oxidation channel for the neutral basic residues relative to the processes that lead to $[M-H]^+$ and $[M-H-NH_3]^+$ species.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Ion/ion reaction between doubly protonated RARARAA²⁺ and IO₄⁻.

Figure 2.

Activation of (a) $[M+2H+IO_4]^+$, (b) $[M+H+O]^+$, (c) $[M-H]^+$, and (d) $[M-H-NH_3]^+$ species derived from the ion/ion reaction between doubly protonated RARARAA and IO_4^- . Degree signs indicate water loss, asterisks indicate ammonia loss, and the lightning bolt is used to indicate species subjected to CID. Red triangles indicate $[b/y-H]^+$ species (i.e., 2 Da lower in mass than expected for unmodified b/y ions). Blue diamonds indicate $[b/y-H-NH_3]^+$ species (i.e., 19 Da lower in mass than expected for unmodified b/y ions).

Figure 3.

Activation of (a) $[M+2H+IO_4]^+$, (b) $[M+H+O]^+$, (c) $[M-H]^+$, and (d) $[M-H-NH_3]^+$ species derived from the ion/ion reaction between doubly protonated KAKAKAA and IO_4^- . Degree signs indicate water loss, asterisks indicate ammonia loss, and the lightning bolt is used to indicate species subjected to CID. Red triangles indicate $[b/y-H]^+$ species (i.e., 2 Da lower in mass than expected for unmodified b/y ions). Blue diamonds indicate $[b/y-H-NH_3]^+$ species (i.e., 19 Da lower in mass than expected for unmodified b/y ions).

Figure 4.

(a) Ion/ion reaction between triply protonated RARARAA and IO_4^- . Activation of (b) $[\text{M}+3\text{H}+2\text{IO}_4]^+$, (c) $[\text{M}+3\text{H}+\text{IO}_4]^{2+}$, and (d) $[\text{M}+2\text{H}+\text{O}]^{2+}$. Degree signs indicate water loss, asterisks indicate ammonia loss, and the lightning bolt is used to indicate species subjected to CID.

Figure 5.

Activation of (a) $[M+2H+IO_4]^+$, (b) $[M+H+O]^+$, (c) $[M-H]^+$, and (d) $[M-H-NH_3]^+$ species derived from the ion/ion reaction between doubly protonated MAP cell-penetrating peptide and IO_4^- . Degree signs indicate water loss, asterisks indicate ammonia loss, and the lightning bolt is used to indicate species subjected to CID. Red triangles indicate $[b/y-H]^+$ species (i.e., 2 Da lower in mass than expected for unmodified b/y ions). Blue diamonds indicate $[b/y-H-NH_3]^+$ species (i.e., 19 Da lower in mass than expected for unmodified b/y ions). Fragments designated by letters and numbers indicate internal fragments; the letter indicates the amino acid and the number indicates its position in the peptide, e.g., A7-K16 indicates an internal fragment containing residues starting at alanine in the seventh position through lysine at the sixteenth position.

Figure 6.

Zoom in of the region around the mass corresponding to ubiquitin $[M+5H]^{5+}$ for (a) DDC CID of ion/ion reaction between ubiquitin $[M+7H]^{7+}$ and IO_4^- , (b) charge-reduction ion/ion reactions between ubiquitin $[M+7H]^{7+}$ and perfluoro-1-octanol anions.

Scheme 1.

Summary of potential oxidation pathways available upon ion/ion reactions with periodate.

a) Generation of $[M-H]^+$ from Lysine b) Generation of $[M-H]^+$ from Arginine **Scheme 2.**

Proposed mechanisms for the generation of $[M-H]^+$ species from neutral basic side-chains upon ion/ion reaction with IO_4^- .

a) Generation of $[M-H-NH_3]^+$ from an N-terminal Lysine b) Generation of $[M-H-NH_3]^+$ from an internal Lysine **Scheme 3.**

Proposed mechanisms for the oxidation of (a) N-terminal and (b) internal lysine residues to $[M-H-NH_3]^+$ species upon ion/ion reaction with IO_4^- .