Fragmentation of Protonated Ions of Peptides Containing Cysteine, Cysteine Sulfinic Acid, and Cysteine Sulfonic Acid

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The oxidation of the sulfhydryl group in cysteine to sulfenic acid, sulfinic acid, and sulfonic acid in proteins is important in a number of enzymatic processes. In this study we examined the fragmentation of four peptides containing cysteine, cysteine sulfinic acid (Cys-SO_2H), and cysteine sulfonic acid (Cys-SO_3H) in an ion-trap mass spectrometer. Our results show that the presence of a Cys-SO_2H in a peptide leads to preferential cleavage of the amide bond at the C-terminal side of the oxidized cysteine residue. The results are important for the determination of the site of the cysteine oxidation and might be useful for the sequencing of cysteine-containing peptides. (J Am Soc Mass Spectrom 2004, 15, 697–702) © 2004 American Society for Mass Spectrometry

Cysteine sulfenic acid (Cys-SOH) is not uncommon [1]; it exists in native proteins, and it can also be introduced via mild oxidation [2, 3]. In the latter, active-site cysteine residues of some enzymes can be selectively oxidized at neutral pH [4–6]. The selective oxidation is due to low pKa for those cysteine residues in the local protein environment, which leads to the formation of cysteine thiolate anion (Cys-S^-). The thiolate anion can readily be oxidized by H_2O_2 to give Cys-SOH [4, 7]. The Cys-SOH is unstable and it reacts with any accessible thiol to form a disulfide or undergoes further oxidation to give cysteine sulfinic acid (Cys-SO_2H) and cysteine sulfonic acid (Cys-SO_3H) [2]. The formation of Cys-SO_2H and Cys-SO_3H has been implicated in the activation of matrix metalloproteinase-7 (MMP-7) [8] and nitrile hydratase [9]. The oxidation to Cys-SO_2H and Cys-SO_3H is generally thought to be irreversible [2]. However, recent studies show that the oxidation of cysteine to Cys-SO_2H in human peroxiredoxin is reversible in vivo [6] and a yeast enzyme that catalyzes this reaction has been identified [10]. In addition, the oxidation of a cysteine residue can yield intramolecular sulfenamide, sulfinamide, and sulfonamide while the cysteine residue is in close proximity to the N-terminus or a lysine residue [11, 12].

Mass spectrometry has been widely used in the structure elucidation of biomolecules including peptides [13–15]. The effect of an acidic amino acid, i.e., aspartic acid, glutamic acid, and Cys-SO_3H, on the fragmentation of protonated peptide ions has been well studied [16–22]. A general conclusion emerging from these studies appears to support that selective cleavage at the C-terminal side of the acidic amino acid residue can occur when the number of ionizing protons does not exceed the number of arginines [16, 20–22]. This is largely attributed to the intramolecular ionic interaction between the side chain of acidic amino acids and the guanidino group of arginine. Non-selective cleavages, however, can occur while the number of ionizing protons exceeds the number of arginine residues in a peptide [16, 20–22]. No systematic study, however, has been reported for the fragmentation of peptides containing a Cys-SO_2H. In this paper we report the fragmentation of protonated ions of peptides containing a cysteine, Cys-SO_2H, or Cys-SO_3H.

Experimental

Peptides used in this study were either purchased from Sigma (St. Louis, MO) or synthesized on an ABI 433 B peptide synthesizer (Applied Biosystems, Foster City, CA) by using standard solid-phase chemistry [23]. Peptides containing an oxidized cysteine residue were prepared by oxidation using Fenton reagents (Fe^{2+}/H_2O_2) and subjected directly to MS/MS analysis or purified by reversed-phase high performance liquid chromatography (HPLC) prior to MS analysis. Under a typical reaction condition, 100-μM peptide solution was incubated at 37 °C in the presence of 0.25 mM $FeSO_4$ and 0.25 mM H_2O_2 for 10–30 min. The reaction was terminated by adding aliquots of methionine solution until its concentration reached 1 mM. For all the sequences studied, we were able to obtain peptides with the cysteine residue being oxidized to Cys-SO_2H and Cys-SO_3H. The combined yield for the formation of

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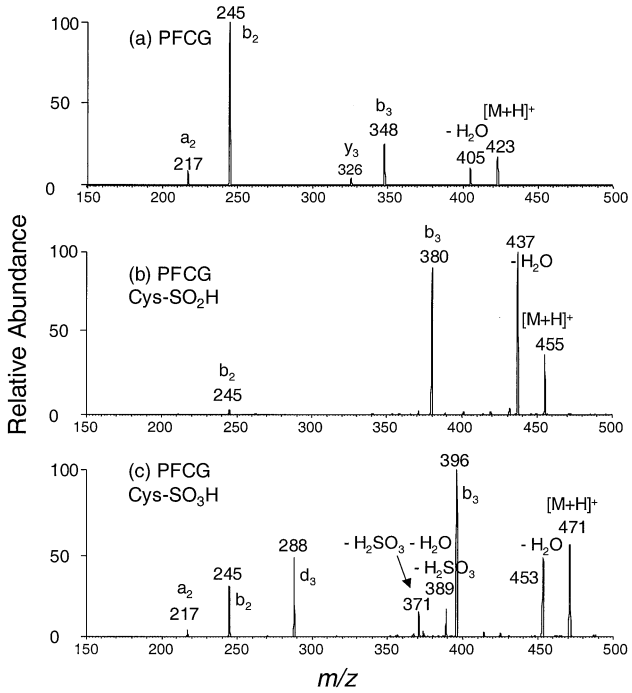


Figure 1. Product-ion spectra of electrospray-produced $[M + H]^+$ ions of PFCG (**a**), PFC(SO_2H)G (**b**), and PFC(SO_3H)G (**c**).

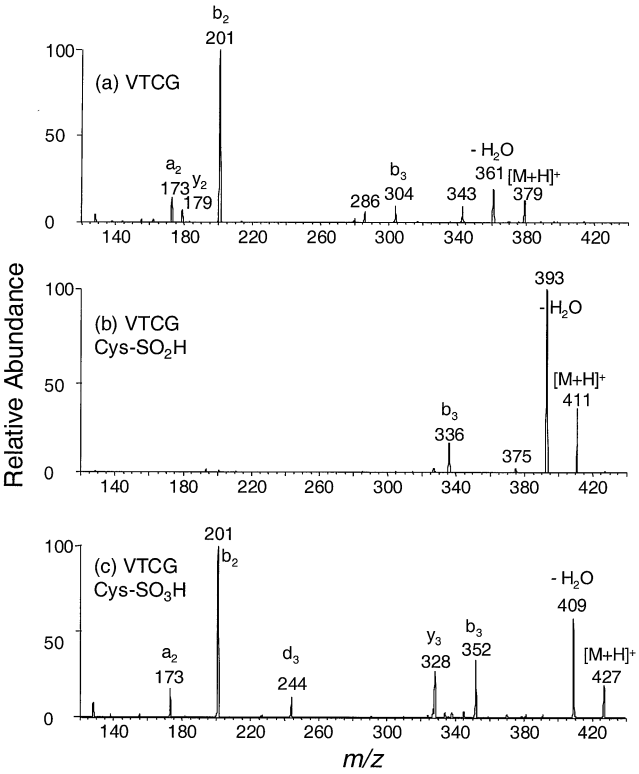


Figure 2. Product-ion spectra of electrospray-produced $[M + H]^+$ ions of VTCG (**a**), VTC(SO_2H)G (**b**), and VTC(SO_3H)G (**c**).

these two oxidation products varied with the sequences of peptides and was estimated to be approximately 20–50% based on peak areas in HPLC trace. It should be noted that the oxidation condition was not rigorously optimized. The amounts of the two oxidation products formed were similar, but could differ by up to 5-fold depending on the sequence of peptide and the oxidation time.

ESI-MS experiments were carried out on an LCQ Deca XP ion-trap mass spectrometer (ThermoFinnigan, San Jose, CA). An equal-volume solvent mixture of acetonitrile and water was used as the carrier and electrospray solvent; a 1-μL aliquot of 10-μM sample solution was injected in each run. The spray voltage was 4.5 kV, and the temperature for the heated capillary was 200 °C. The automated gain control (AGC) feature was employed in MS and MS/MS, and the maximum numbers of ions for these modes were set to be 5×10^7 and 2×10^7, respectively. The mass width for precursor ion selection in MS/MS was 2 *m/z*, and the normalized collisional energy was optimized (18–25%) so that the precursor ion is clearly present but not the most abundant in the product-ion spectrum. Each spectrum was obtained by averaging approximately 50 scans, and the time for each scan was 0.1 s.

Results and Discussion

PFCG, PFC(SO_2H)G and PFC(SO_3H)G

The product-ion spectrum of the ESI-produced $[M + H]^+$ ion of PFCG shows an abundant b_2 ion of *m/z* 245 (Figure 1a). While the cysteine (Cys) is oxidized to Cys-SO_2H, the product-ion spectrum gives a much less abundant b_2 ion. Water loss and cleavage at the C-terminal side of the Cys-SO_2H, however, become very facile, which give an ion of *m/z* 437 and a b_3 ion, respectively (Figure 1b). Similarly, the product-ion spectrum of the ESI-produced $[M + H]^+$ ion of PFC(SO_3H)G shows that the formation of $[M + H - H_2O]^+$ and b_3 ions occurs readily. The b_2 ion, however, is still produced abundantly (Figure 1c).

VTCG, VTC(SO_2H)G, and VTC(SO_3H)G

Similar to PFCG, the product-ion spectrum of the $[M + H]^+$ ion of VTCG shows the formation of an abundant b_2 ion (Figure 2a). The oxidation of the Cys residue to Cys-SO_2H and Cys-SO_3H again leads to the facile formation of a b_3 ion (Figure 2b and c). It is worth noting that the enhancement for the formation of the b_3 ion is relatively more for VTC(SO_2H)G than for VTC(SO_3H)G because the b_3 ion is the only fragment ion other than the water loss fragment observed in the product-ion spectrum for the former, whereas the b_3 ion is produced with similar abundance as other fragment ions, i.e., a_2, b_2, and y_3, in that of the latter (Figure 2b and c). This conclusion seems to be also valid for the PFCG with the Cys being oxidized to Cys-SO_2H and Cys-SO_3H (vide supra).

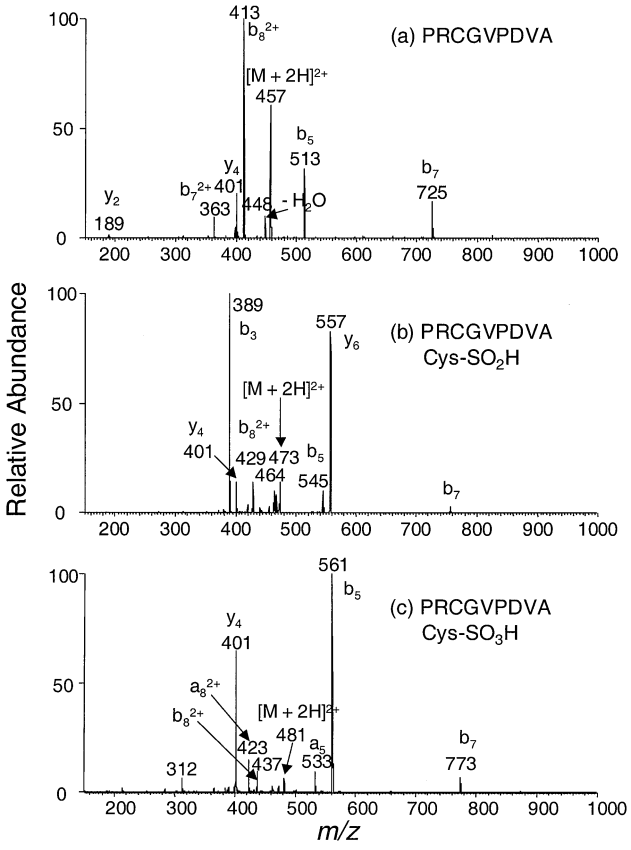


Figure 3. Product-ion spectra of electrospray-produced $[M + 2H]^{2+}$ ions of PRCGVPDVA (**a**), PRC(SO_2H)GVPDVA (**b**), and PRC(SO_3H)GVPDVA (**c**).

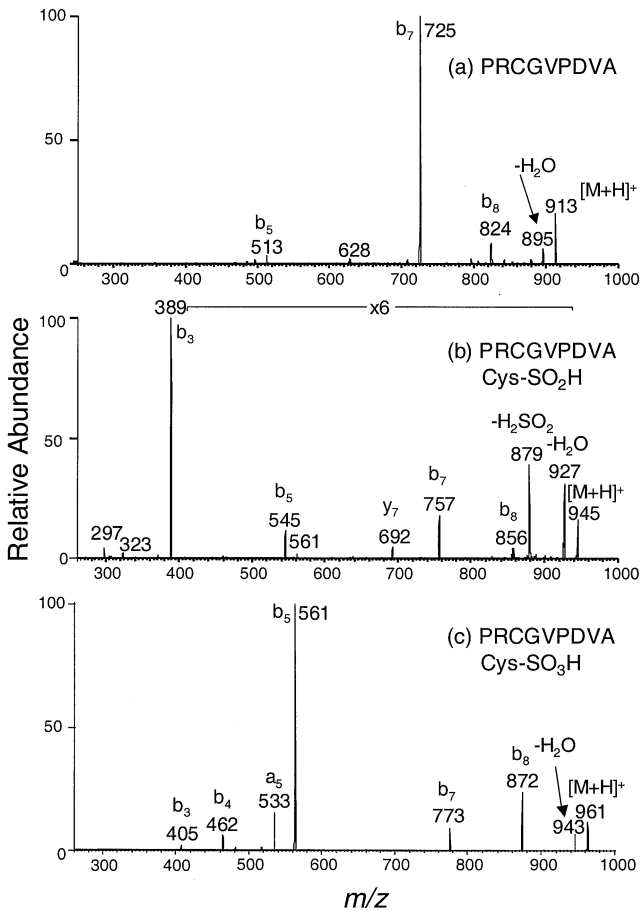


Figure 4. Product-ion spectra of electrospray-produced $[M + H]^{+}$ ions of PRCGVPDVA (**a**), PRC(SO_2H)GVPDVA (**b**), and PRC(SO_3H)GVPDVA (**c**).

PRCGVPDVA, PRC(SO_2H)GVPDVA, and PRC(SO_3H)GVPDVA

The above results were obtained with short peptides where singly-charged precursor ions were used for collisional activation. It is important to demonstrate whether the characteristic cleavages at the C-terminal side of the Cys-SO_2H and Cys-SO_3H also occur in longer peptides. To this end, we oxidized PRCGVPDVA and RGDGGGCR by Fenton reagents (Fe^{2+}/H_2O_2) and separated the mixture by reverse-phase HPLC. The former peptide is part of the cysteine switch domain of matrix metalloproteinase-7 (MMP-7), and it has been shown that HOCl can oxidize the cysteine residue in this peptide to Cys-SO_2H and Cys-SO_3H [8]. Similarly, we found that the Fenton reagents can oxidize the cysteine residue to yield Cys-SO_2H and Cys-SO_3H.

The product-ion spectrum of the ESI-produced $[M + 2H]^{2+}$ ion of the unmodified peptide shows the formation of y_2, y_4, and their complementary b_7 and b_5 ions (Figure 3a). In addition, we observed an abundant b_8^{2+} ion. The product-ion spectrum changes quite obviously while a Cys-SO_2H residue replaces the Cys residue. The most distinctive alteration is the production of an abundant b_3 and its complementary y_6 ions (Figure 3b). Again the formation of these two ions requires cleavage at the C-terminal side of the Cys-SO_2H residue. The b_3 and y_6 ions, however, are almost undetectable in the MS/MS of the $[M + 2H]^{2+}$ ion of the peptide containing a Cys-SO_3H (Figure 3c). Comparing to the product-ion spectrum of the $[M + 2H]^{2+}$ ion of the unmodified peptide, that of the peptide containing the Cys-SO_3H residue gives more abundant b_5 and y_4 ions. It is worth noting the b_5 and y_4 ions were not produced abundantly in the product-ion spectrum of the $[M + 2H]^{2+}$ ion of the peptide bearing a Cys-SO_2H. We attribute this to the presence of an energetically more favorable cleavage pathway, i.e., the production of b_3 and y_6 ions, in the fragmentation of the $[M + 2H]^{2+}$ ion of the latter peptide.

For comparison, we also acquired the product-ion spectra of the $[M + H]^{+}$ ions of PRCGVPDVA, PRC(SO_2H)GVPDVA, and PRC(SO_3H)GVPDVA (Figure 4). In contrast to the product-ion spectra of the doubly-charged precursors, we did not observe abundant y ions in those of the singly-charged precursors (Figures 3 and 4). This is likely due to the fact that the arginine residue, which is the most favorable site for protonation in this peptide, is in close proximity to the

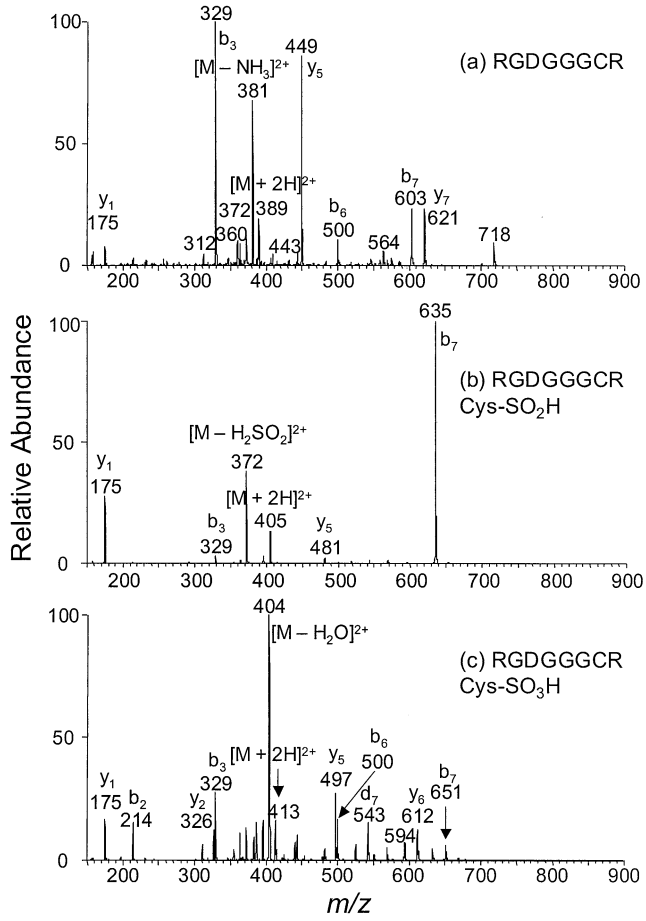


Figure 5. Product-ion spectra of electrospray-produced $[M + 2H]^{2+}$ ions of RGDGGGCR (**a**), RGDGGGC(SO_2H)R (**b**), and RGDGGGC(SO_3H)R (**c**).

N-terminus; fragments bearing the C-terminus of the peptide, therefore, are unlikely to be protonated. Moreover, the presence of a Cys-SO_2H again results in facile cleavage of the amide bond at the C-terminal side of Cys-SO_2H to give a dominant b_3 ion (Figure 4b). In contrast, the product-ion spectrum of the $[M + H]^+$ ion of PRC(SO_3H)GVPDVA shows that the most abundant cleavage occurs at N-terminal side of proline to give a b_5 ion. The b_3 ion (*m/z* 405) is also produced, but in very low abundance (Figure 4c).

Tsaprailis et al. [21] reported that collisional activation of protonated peptide ions containing arginine and aspartic acid or glutamic acid residues results in selective cleavages at the C-terminal side of the acidic residues if the number of ionizing protons does not exceed the number of arginine residues. Our data with the $[M + H]^+$ ion of the PRCGVPDVA appear not to be consistent with this view because the selective cleavage occurs at the amide bond between valine and proline, which gives the b_5 ion. However, it has been recently shown that cleavage N-terminal to a proline residue is a very facile process especially when the residue at the N-terminal side of the proline is a valine [24, 25]. It seems that sequence context plays a more important

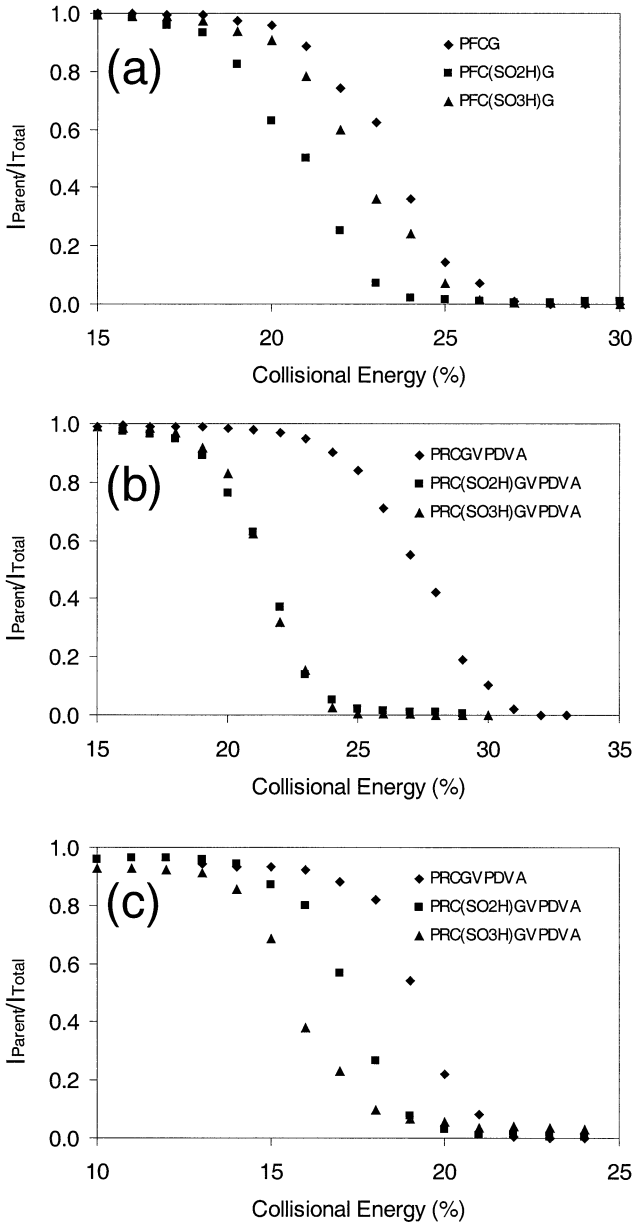
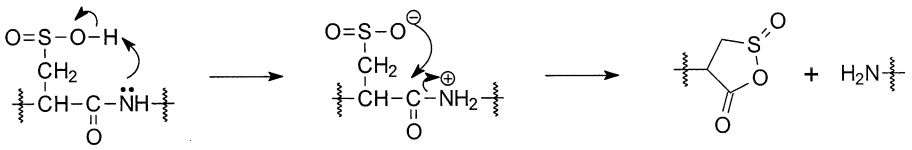


Figure 6. A plot of the fraction of parent ion intensity (I_{parent}/I_{total}) versus the collisional energy for the fragmentation of the $[M + H]^+$ ions of PFCG, PFC(SO_2H)G, and PFC(SO_3H)G (**a**). Similar plots for the fragmentation of the $[M + 2H]^{2+}$ (**b**) and $[M + H]^+$ (**c**) ions of PRCGVPDVA, PRC(SO_2H)GVPDVA, and PRC(SO_3H)GVPDVA.

role in the fragmentation of the $[M + H]^+$ ion of PRC(SO_3H)GVPDVA than intramolecular ionic interaction between the side chains of acidic and basic amino acid residues.

RGDGGGCR, RGDGGGC(SO_2H)R, and RGDGGGC(SO_3H)R

We also acquired the product-ion spectra of the $[M + 2H]^{2+}$ and $[M + H]^+$ ions of RGDGGGCR, RGDGGGC(SO_2H)R, and RGDGGGC(SO_3H)R, and the results are similar to the other three sets of peptides



discussed above. In the product-ion spectrum of the $[M + 2H]^{2+}$ ion of the unmodified peptide, we observed facile cleavage at the C-terminal side of aspartic acid with the formation of abundant b_3 and y_5 ions, though cleavages at other sites were also observed (Figure 5a). The oxidation of cysteine to Cys-SO_2H results in marked change of the product-ion spectrum; cleavage occurs readily at the C-terminal side of the modified cysteine and it gives abundant b_7 and y_1 ions (Figure 5b). Cleavage at the C-terminal side of aspartic acid can also occur, but the abundances of the resulting b_3 and y_5 ions are much lower than those for the b_7 and y_1 ions (Figure 5b). In contrast, the replacement of cysteine with Cys-SO_3H does not give rise to enhanced cleavage at the C-terminal side of Cys-SO_3H (Figure 5c). The product-ion spectra of the $[M + H]^+$ ions gave similar results (data not shown).

"Break-down" Curves for the Fragmentations

The facile cleavage at the C-terminal side of the Cys-SO_2H may imply that the collisional energy required for the fragmentation of Cys-SO_2H-bearing peptides is lower than that of the unmodified peptides. To test this we acquired the product-ion spectra at different collisional energies and plotted the fraction of the parent ion intensity in total ion intensity versus the collisional energies ("break-down" curve).

The "break-down" curves (Figure 6) show that the oxidation of the Cys residue to Cys-SO_2H or Cys-SO_3H makes the peptides more susceptible to fragmentation. The relative ease for the fragmentation of peptides containing Cys-SO_2H and Cys-SO_3H depends on both the sequence of the peptide and the number of protons present in the peptide ion. In this respect, the fragmentation of the $[M + H]^+$ ion of PFC(SO_2H)G requires lower collisional energy than that of PFC(SO_3H)G (Figure 6a). Whereas the fragmentation of the $[M + 2H]^{2+}$ ions of PRC(SO_2H)GVPDVA and PRC(SO_3H)GVPDVA requires similar collisional energy, the fragmentation of the $[M + H]^+$ ion of PRC(SO_2H)GVPDVA needs lower collisional energy for fragmentation than that of PRC(SO_3H)GVPDVA (Figure 6b and c).

Conclusions

In the presence of Fenton reagents, the cysteine residue in a peptide can be oxidized to Cys-SO_2H and Cys-SO_3H. Collisional activation of the protonated ions of those peptides leads to facile cleavage(s) at the C-terminal side of the Cys-SO_2H to give the corresponding b and/or y ions. In addition, this cleavage is more facile for peptides containing a Cys-SO_2H than for those bearing a Cys-SO_3H.

This observation appears to be general. In addition to the peptides studied in this paper, facile cleavage was observed at the C-terminal side of Cys-SO_2H in a peptide C(SO_2H)GVPDVAE [8]. Similarly, the y_6 ion is one of the most abundant ions observed in the product-ion spectrum of the $[M + 3H]^{3+}$ ion of a peptide ESGSLSPEHGPVVVHC(SO_2H)SAGIGR, and the formation of the y_6 ion requires cleavage at the C-terminal side of the Cys-SO_2H [26]. However, the y_6 ion is much less abundant in the product-ion spectrum of the $[M + 3H]^{3+}$ ion of the same peptide with the cysteine being oxidized to Cys-SO_3H [26]. Moreover, we demonstrated that the protonated ions of peptides containing a Cys-SO_2H require lower energy for the fragmentation than those bearing an unmodified cysteine.

Similar to the mechanism proposed for the cleavage of the amide bond at the C-terminal side of an aspartic acid [17], we proposed a mechanism for the cleavage at the amide bond that is C-terminal to the Cys-SO_2H (Scheme **1**). The preferential cleavage of the amide bond at the C-terminal side of the Cys-SO_2H provides information for determining the site of cysteine oxidation. This, in combination with the facile formation of Cys-SO_2H from Cys, also affords a convenient way for the identification and localization of cysteine residue in a peptide.

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