Collisionally-Activated Dissociation of Peptides with a Disulfide Bond: Confirmation of the Mobile-Proton Model Based Explanation

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Abstract: In the present study, collisionally-activated dissociation (CAD) experiments were performed under low energy collision conditions in six peptides containing a disulfide bond. Fragments produced as a result of the cleavage of a disulfide bond were obtained after CAD in four peptides (bactenecin, TGF-α, cortistantin, and linearly linked peptide, Scheme 1) with basic amino acid residues. In contrast, the CAD analysis of two peptides with no basic residue (oxytocin and tocinoic acid) rarely produced fragments indicative of cleavage of a disulfide bond. These results are consistent with the mobile proton model suggested by the McLuckey and O'Hair groups (ref. 22 and 23); nonmobile protons sequestered at basic amino acid residues appear to promote the cleavage of disulfide bonds.

Key words: Disulfide bond, Peptides, Collisionally activated dissociation (CAD), Mobile proton model, Tandem mass spectrometry

Introduction

A disulfide bond is a posttranslational modification (PTM) that plays a critical role in stabilizing the native conformations of a large number of proteins.[1] In general, disulfide bonds (R-S–S-R) are chemically reduced to sulfhydryl ends and then alkylated prior to mass spectrometry analysis because disulfide bond cleavage is not readily observed when low-energy collision dissociation methods are used.[2–5] A variety of efforts have been made to design mass spectrometry-based strategies that do not require these chemical reduction steps and that directly cleave disulfide bonds.

For example, the appropriate matrix choice for UV-MALDI (337 nm) has been shown to result in cleavage of disulfide bonds during in-source decay.[6] Zubarev and coworkers also demonstrated that UV photodissociation at 157 nm could cleave both inter- and intramolecular disulfide bonds.[7] UV absorption at the S–S bond was presumed to lead to disulfide bond cleavage through dissociative electronic transition followed by H-atom rearrangements. In contrast, collisionally-activated dissociation (CAD) of multiply charged anions revealed a strong preference for cleavage of disulfide bonds compared to other bond types[8,9] due to the exclusive dissociation of the side chains of cysteine residues under low-energy CAD conditions.[10,11] Other methods commonly used to selectively cleave disulfide bonds are electron capture dissociation (ECD), electron transfer dissociation (ETD), and electron detachment dissociation (EDD).[12–16] McLafferty *et al*. showed that ECD of multiply charged cations resulted in the selective cleavage of disulfide bonds, even allowing for the determination of the locations of two disulfide bonds among ten potential S–S pairs in a viral prolyl 4-hydroxylase.[8,12,13] Recently, our group also showed that a free radical generated by the release of a TEMPO group upon collisional activation of a TEMPO-Bz-coupled peptide could effectively cleave a disulfide bond (S–S) or its neighboring C–S bond.[17] Alternatively, transition, alkali, and alkaline earth metal complexes of peptides have been used as gas phase disulfide bond cleavage reagents.[18–20]

However, only a few studies have investigated disulfide bond cleavage using low-energy CAD of protonated cations.[21–23] Under ion trap collisional activation conditions, McLuckey *et al*. observed that disulfide cleavage was strongly influenced by the charge states of the protonated precursors.[22] Singly protonated ions were found to fragment almost exclusively at disulfide bonds, whereas highly protonated ions fragmented exclusively at the peptide backbone outside the cyclic region formed by a disulfide bond. O'Hair *et al.* noted that the absence of a mobile proton facilitated disulfide bond cleavage during low-energy collision activation of singly protonated peptides.[23,24] When a mobile proton is absent, more energy is required for backbone dissociation than for disulfide bond cleavage.

The mobile proton model outlined above is the prevailing explanation for the preferred disulfide bond cleavage of low protonated states under low-energy collision activation conditions.[23,24] In the present study, we carried out ion-trap low-energy CAD experiments using six disulfide bond-containing peptides, four peptides with basic amino-acid residues such

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as arginine (R) and lysine (K) and two peptides without R or K residue (Scheme 1), to determine if the mobile proton model is valid.

Experimental

Experiments were performed on an ion-trap mass spectrometer (LCQ Deca, Finnigan, CA, USA). Detection of ions and acquisition of data were performed in the positive ion mode.

Peptide	Sequence
Bactenecin	RLCRIVVIRVCR
TGF-α	CHSGYVGVRC
Cortistantin	PCKNFFWKTFSSCK
Linearly linked Peptide	CFIR (Sequence 1) Ac-NCPR (Sequence 2)
Oxytocin	CYIQNCPLG
Tocinoic acid	CYIQNC

Scheme 1. Sequences of six peptides with a disulfide bond. Disulfide bonds are shown as lines connecting two cysteine residues. Basic amino acid residues such as arginine (R) and lysine (K) are denoted with a box.

The peptides of interest were dissolved in a 49:49:2 (v/v/v) H_2O:CH_3OH:CH_3COOH solution and prepared at a concentration of 20 μM. The prepared samples were directly infused through an electrospray ionization (ESI, voltage = +4.0 kV) source at the flow rate of 2–3 μL/min. Tandem mass spectra were obtained with low-energy CAD. Mass spectrometry parameters were capillary temperature, 200 °C; tube lens off-set voltage, 0–30 V; sheath gas flow rate (arb), 20; normalized collision energy, 25-35%; isolation width, 2 Da; and capillary voltage, 20 V. Mass spectra for each peptide were acquired by averaging 20 scanned spectra.

The peptides shown in Scheme 1 are commercially available (Bachem, Seoul, Korea), except for the linearly linked peptide that was custom synthesized (Anygen, Gwangju, Korea). Peptides were used without further purification, and all solvents were of HPLC grade.

Results and Discussion

A solution of a peptide of interest was electrosprayed and detected in the positive ion mode. The most abundant precursor charge state of a peptide in the ESI mass spectrum was isolated and subjected to CAD.

Peptides with basic amino acid residue(s)

Figure 1 shows the CAD mass spectra for bactenecin, TGF-α, cortistantin, and the linearly linked peptide, all of which contain at least one basic amino acid residue. For

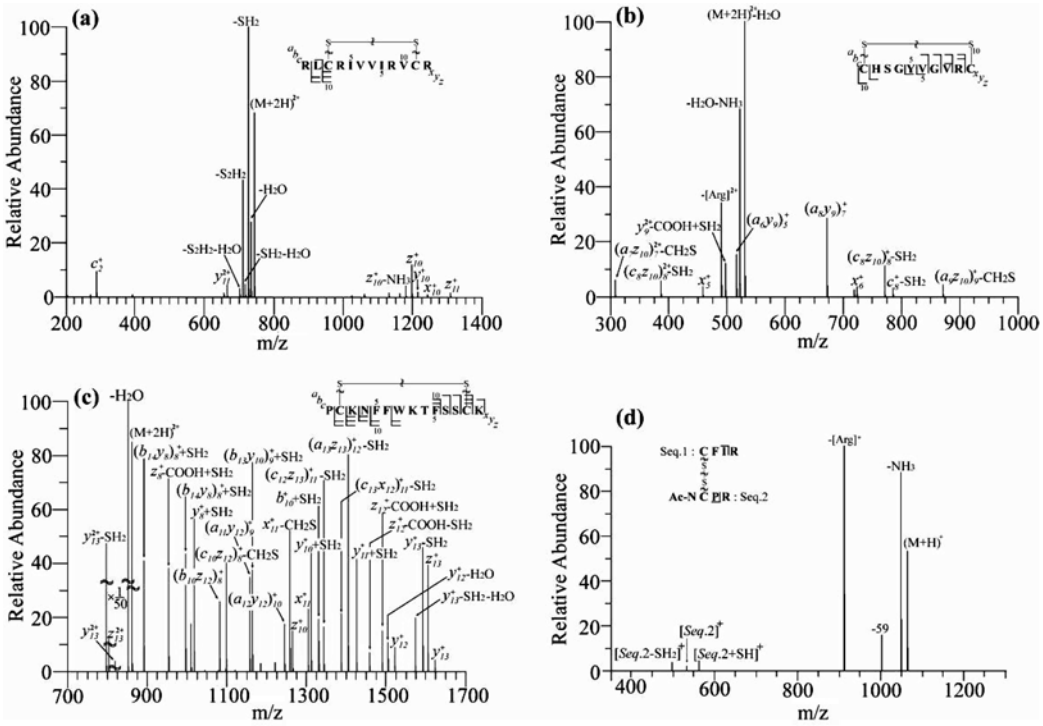

Figure 1. CAD MS/MS mass spectra for bactenecin (a), TGF-α (b), cortistantin (c), and a linearly linked peptide (d) protonated cations. For clarity, the mass spectrum for cortistantin (c) is displayed in red.

bactenecin, which has four arginines, major peaks in the CAD spectrum of doubly-protonated precursor ions, $(M+2H)^{2+}$, arose from neutral molecule loss of H_2O, H_2S, or H_2S_2 (Figure 1a). In addition, a number of peptide backbone fragments were observed; for example, c_2^+, z_{10}^+-NH_3, z_{10}^+, y_{10}^+, x_{10}^+, and z_{11}^+, which were produced by backbone dissociation outside the cyclic region defined by the disulfide bond. Among these fragments, peaks arising from the neutral molecule loss of SH_2 or S_2H_2 are of interest because these peaks clearly indicate that cleavage of S–S or C–S bonds occurred readily during CAD of doubly-protonated molecular ions, although these fragmentations were not followed by peptide backbone dissociations that would be useful for peptide sequencing.

For TGF-α, which has a single arginine residue, more extensive peptide backbone fragmentation was observed (Figure 1b). All of the generated fragments, except for –[Arg]$^{2+}$, $-H_2O$, and $-H_2O-NH_3$, could be explained by cleavage of a disulfide bond or its neighboring C–S bond, in addition to cleavage of one or two backbone bonds. For example, $(a_8y_9)_7^+$ at *m/z* 672.1 must have been generated by cleavage of the inter-residue bonds 8 (V–R for a_8) and 1 (C–H for y_9), in combination with the rupture of the disulfide bond between Cys1 and Cys10. The subscript 7 in $(a_8y_9)_7^+$ indicates the length of amino acid residues in this fragment. The extended backbone dissociations for TGF-α compared with those of bactenecin can be understood in terms of the mobile proton model (24). As mentioned, TGF-α has only one arginine residue. For doubly protonated TGF-α cations, it is likely that one proton was sequestered at the basic arginine residue and the other proton was rather loosely bound, in contrast to bactenecin with four arginine residues, resulting in more peptide backbone dissociations in CAD for TGF-α than for bactenecin.

The CAD results of doubly protonated cortistantin, which has three lysines, showed an even more extensive fragmentations pattern than that of TGF-α (see Figure 1c). A very large number of fragments were observed in the MS/MS mass spectrum, and most of the observed fragments were due to cleavage of the disulfide bond between Cys2 and Cys13 or the neighboring C–S bond in combination with single or double backbone dissociations. We also performed CAD for a peptide linearly linked by a disulfide bond (see Scheme 1 and Figure 1d, in which a number of fragments were produced due to loss of NH_3, 59 Da, or an arginine residue. More interestingly, peaks were present at *m/z* 497.5, 531.5, and 563.4, corresponding to [Seq. 2–SH_2]$^+$, [Seq. 2]$^+$, and [Seq. 2 + SH]$^+$, respectively; fragments which could only have been generated by cleavage of an S–S or C–S bond.

These CAD results for the four peptides that contain basic amino acids clearly indicate that disulfide bonds or adjacent C-S bonds are readily cleaved under low energy ion-trap CAD conditions. These results are in good agreement with the mobile proton hypothesis of the McLuckey and O'Hair groups.[22,23]

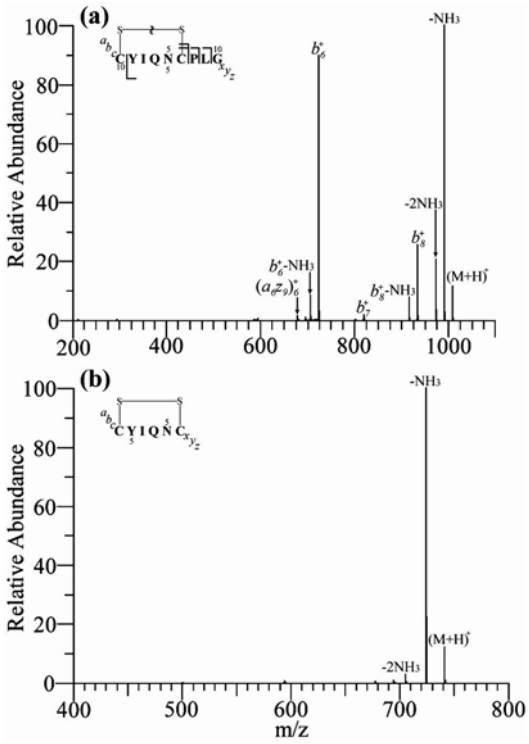

Figure 2. CAD MS/MS mass spectra for oxytocin (a) and tocinoic acid (b) protonated cations.

Peptides without a basic amino acid residue

Oxytocin and tocinoic acid, which do not contain arginine or lysine, were also subjected to CAD. Figures 2a and b show the CAD mass spectra for singly protonated oxytocin and tocinoic acid ions, respectively. In Figure 2a, the major fragments resulted from NH_3 loss and b_6^+. Additionally, b_7^+, b_8^+, and their NH_3 loss peaks were observed in relatively low abundance. These backbone fragments were generated by cleavage of the backbone outside the cyclic region formed by the disulfide bond between Cys1 and Cys6. There is observed no fragment for which peptide backbone cleavage occurred inside the cyclic region formed by a disulfide bond, except for a very low abundant $(a_6z_9)_6^+$ peak that was also generated by cleavage of the inter-residue bond 6 and 1 in addition to the disulfide bond. The dissociation of a peptide backbone between Cys1 and Cys6 is presumed to occur as likely as the backbone outside the cyclic region does. However, if this dissociation was not accompanied by disulfide bond cleavage, fragments indicating the dissociation between Cys1 and Cys6 cannot be observed in the resulting MS/MS spectrum. For tocinoic acid, only peaks arising from the loss of NH_3 were found (Figure 2b); no fragment peak indicating the cleavage of a disulfide bond was present.

Nonmobile protons promote the cleavage of a disulfide bond

The above CAD results are in good agreement with the

mobile proton model.[22–24] For peptides without a basic residue, protons are not sequestered; in other words, protons are mobilized and catalyze peptide backbone dissociations. As a result, the cleavage of a disulfide bond becomes relatively much more difficult. In contrast, in the case of peptides with a basic residue, protons are strongly sequestered. As a result, cleavage of a disulfide bond or its adjacent C–S bond is favorable, so fragments generated via cleavage of a disulfide bond or its neighboring bond are ubiquitous.

To summarize, our experimental results confirm the mobile proton model-based explanation for the contrasting disulfide bond cleavage results observed for peptides with and without basic residue. Greater understanding of the factor governing disulfide bond cleavage will facilitate future development of strategies that do not require the cumbersome disulfide bond chemical reduction procedure.

Acknowledgments

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References

1. Matsumura, M.; Signor, G.; Matthews, B. W. *Nature*, **1989**, 342, 2913.
2. Loo, J. A.; Edmonds, C. G.; Udseth, H. R.; Smith, R. D. *Anal. Chem.* **1990**, 62, 693.
3. Gunawardena, H. P.; O'Hair, R. A. J.; McLuckey, S. A. *J. Proteome Res.* **2006**, 5, 2087.
4. Stephenson, J. L. Jr.; Cargile, B. L.; McLuckey, S. A. *Rapid Commun. Mass Spectrom.* **1999**, 13, 2040.
5. Gorman, J. J.; Wallis, T. P.; Pitt, J. J. *Mass Spectrom. Rev.* **2002**, 21, 183.
6. Huwiler, K. G.; Mosher, D. F.; Vestling, M. M. *J. Biomol. Technol.* **2003**, 14, 289.
7. Fung, E. Y. M.; Kjeldsen, F.; Silivra, O. A.; Chan, D. T. W.; Zubarev, R. A. *Angew. Chem.* **2005**, 117, 6557.
8. Chrisman, P. A.; McLuckey, S. A. *J. Proteome Res.* **2002**, 1, 549.
9. Zhang, Mingxuan; Kaltashov, I. A. *Anal. Chem.* **2006**, 78, 4820.
10. Bowie, J. H.; Brinkworth, C. S.; Dua, S. *Mass Spectrom. Rev.* **2002**, 21, 87.
11. Bilusich, D.; Brinkworth, C. S.; McAnoy, A. M.; Bowie, J. H. *Rapid Commun. Mass Spectrom.* **2003**, 17, 2488.
12. Zubarev, R. A.; Kruger, N. A.; Fridriksson, M. A.; Lewis, M. A.; Horn, D. M.; Carpenter, B. K.; McLafferty, F. W. *J. Am. Chem. Soc.* **1999**, 121, 2857.
13. Ge, Y.; Lawhorn, B. G.; ElNaggar, M.; Strauss, E.; Park, J.-H.; Begley, T. P.; McLafferty, F. W. *J. Am. Chem. Soc.* **2002**, 124, 672.
14. Syka, J. E.; Coon, J. J.; Schroeder, M. J.; Shabanowitz, J.; Hunt, D. F. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, 101, 9528.
15. Chrisman, P. A.; Pitteri, S. J.; Hogan, J. M.; McLuckey, S. A. *J. Am. Soc. Mass Spectrom.* **2005**, 16, 1020.
16. Kalli, A.; Håkansson, K. *Int. J. Mass Spectrom.* **2007**, 263, 71.
17. Lee, M.; Kang, M.; Moon, B.; Oh, H. B. *Analyst* **2009**, 134, 1706.
18. Mihalca, R.; van der Burgt, Y. E. M.; Heck, A. J. R.; Heeren, R. M. A. *J. Mass Spectrom.* **2007**, 42, 450.
19. Lioe, H.; Duan, M.; O'Hair, R. A. J. *Rapid Commun. Mass Spectrom.* **2007**, 21, 2727.
20. Kim, H. I.; Beauchamp, J. L. *J. Am. Chem. Soc.* **2008**, 130, 1245.
21. Jones, M. D.; Patterson, S. D.; Lu, H. S. *Anal. Chem.* **1998,** 70, 136.
22. Wells, J. M.; Stephenson, J. L. Jr.; McLuckey, S. A. *Int. J. Mass Spectrom.* **2000**, 203, A1.
23. Lioe, H.; O'Hair, R. A. J. *J. Am. Soc. Mass Spectrom.* **2007**, 18, 1109.
24. Wysocky, V. H.; Tsaprailis, G.; Smith, L. L.; Breci, L. A. *J. Mass Spectrom.* **2000**, 35, 1399.