Methionine oxidation plays a critical role in many processes of biologic and biomedical importance, including cellular redox responses and stability of protein pharmaceuticals. Bottom-up methods for analysis of methionine oxidation can suffer from incomplete sequence coverage, as well as an inability to readily detect correlated oxidation between 2 or more methionines. However, the methodology for quantifying protein oxidation in top-down analyses is lacking. Previous work has shown that electron transfer dissociation (ETD)-based tandem mass spectrometry (MS/MS) fragmentation offers accurate and precise quantification of amino acid oxidation in peptides, even in complex samples. However, the ability of ETD-based MS/MS fragmentation to accurately quantify amino acid oxidation of proteins in a top-down manner has not been reported. Using apomyoglobin and calmodulin as model proteins, we partially converted methionines into methionine sulfoxide by incubation in H₂O₂. Using top-down ETD-based fragmentation, we quantified the amount of oxidation of various ETD product ions and compared the quantified values with those from traditional bottom-up analysis. We find that overall quantification of methionine oxidation by top-down MS/MS ranges from good agreement with traditional bottom-up methods to vast differences between the 2 techniques, including missing oxidized product ions and large differences in measured oxidation quantities. Care must be taken in transitioning ETD-based quantitation of oxidation from the peptide level to the intact protein level.

**Key Words:** proteomics, post-translational modification, hydroxyl radical protein footprinting

**INTRODUCTION**

Proteins are sensitive to oxidative damage, which can lead to disturbances in cellular and tissue homeostasis. Protein oxidation is also known as a contributory factor in numerous diseases.¹ The sulfur-containing amino acids cysteine and methionine are most susceptible to the reactive oxygen species commonly generated in biologic systems.² Among them, methionine (Met) residues play important roles as antioxidant defense and regulation of cellular function via reversible oxidation and reduction.³⁻⁵ In the presence of reactive oxygen species, Met readily forms methionine sulfoxide (MetSO) by addition of oxygen to its sulfur⁶; the conversion of MetSO to methionine sulfone is much slower. In last few decades proteins and peptides have become important therapeutic agents for various diseases.¹¹ However, the rise of biopharmaceuticals has brought a new need for accurate measurement of protein modifications, including oxidation, because the chemical stability of proteins is important in development and storage.¹² Oxidation, particularly of Met, can lead to changes in secondary, tertiary, and quaternary structure.¹³, ¹⁴ Similarly, oxidation of methionine is a known major degradation pathway of purified proteins and is of major biomedical and economic importance in the development of protein pharmaceuticals such as monoclonal antibodies. Methionine oxidation occurs in pharmaceutical protein formulations during processing and storage and can be induced by presence of transition metal ions, contaminating oxidants, pH, temperature, buffer composition, and light. Accurate quantification of MetSO is challenging,¹⁵, ¹⁶ and standard bottom-up methods are unable to detect correlated oxidation between Met residues. Correlation in methionine oxidation is crucial because it helps indicate mechanisms of oxidation-induced conformational change.¹⁷ Our group has previously shown that electron transfer dissociation (ETD) fragmentation is able to quantify oxidation of various amino acids, including methionine oxidation, in a peptide context based on the ratio of oxidized vs. unoxidized product ion abundance; the oxidation event does not alter the ETD fragmentation pathway.¹⁸, ¹⁹ However, this measurement has only been made in peptides. ETD fragmentation of proteins can be influenced by other factors, including structural features.²⁰ It is unknown if quantification of amino acid oxidation by ETD product ion abundance within an intact protein will be accurate. In the present study, we investigate the accuracy and precision of top-down ETD tandem mass spectrometry (MS/MS) to quantify MetSO formation, regarded to be the most labile common protein oxidation product in the gas phase.²¹, ²² To test the accuracy and precision of top-down ETD MS/MS for quantitation of MetSO formation, we examined the incomplete oxidation of myoglobin and calmodulin by
hydrogen peroxide. Myoglobin contains 2 methionines: one closer to the N terminus and one closer to the C terminus. Calmodulin is a much more complex model system, with 9 methionines. By comparing the quantitation of oxidation of those methionines by both traditional bottom-up methods and top-down fragmentation using ETD, we test the accuracy of both c- and z-ion series for measurement of protein oxidation products.

**EXPERIMENTAL SECTION**

**Materials and methods**

Apomyoglobin from equine skeletal muscle, calmodulin from bovine testes, formic acid, propylene carbonate and ammonium bicarbonate were purchased from Millipore-Sigma (Burlington, MA, USA). Thirty percent hydrogen peroxide was purchased from J. T. Baker (Thermo Fisher Scientific, Waltham, MA, USA). Liquid chromatography (LC)–mass spectrometry (MS) grade acetonitrile (ACN) and water were purchased from Thermo Fisher Scientific. DTT was purchased from Soltech Ventures (Beverly, MA, USA). Sequencing-grade modified trypsin was purchased from Promega (Madison, WI, USA).

**Sample preparation**

Five hundred microliters of 1 mM of protein was incubated with 100 mM H₂O₂, shielded from UV light. The reaction with apomyoglobin was allowed to proceed for 6 h, whereas the reaction with calmodulin was allowed to proceed for 1 h. The reaction was halted by buffer exchange through a 5 kDa MW cutoff filter (Sartorious, Göttingen, Germany). A sample (50 µl) was set aside for bottom-up analysis. The remaining supernatant was dissolved in 50% ACN, 0.1% formic acid, and 2% propylene carbonate supercharging reagent for top-down analysis.

**Top-down analysis**

Sample was injected into a Thermo Orbitrap Fusion Tribrid (Thermo Fisher Scientific) by direct infusion at 5 µl/min in positive mode with a spray voltage 3200, capillary temperature 300°C at a nominal Orbitrap resolution of 120,000. ETD fragmentation was performed with an isolation window of 10 m/z units to capture all protein oxidation products. ETD reaction times ranged from 10 to 40 ms at an automatic gain control target of 5.0 × 10⁴. We found that a reaction time of 25 ms gave superior sequence coverage for all apomyoglobin, whereas an ETD reaction time of 15 ms gave superior coverage for calmodulin; all ETD data presented are for a 25-ms reaction time for apomyoglobin and 15 ms for calmodulin with electron-transfer–higher-energy collision dissociation collision energy set to 5 to provide supplemental activation. Altered ETD parameters altered the product ions identified but resulted in substantially lower sequence coverage, poorer product ion abundance, and insufficient product ions for assignment of methionine oxidation sites (unpublished results). Direct infusion acquisitions were acquired for 10 min. The 10 min acquisition was split into two 5-min time windows for spectra summation, and these 2 summed spectra were used as technical duplicates for statistical analysis. ProSight Lite was used for initial sequence coverage analysis using a signal-to-noise threshold of 3, resolution of 120,000, a fit factor of 80, and remainder of 25, but all data were ultimately analyzed and annotated manually both to validate automated product ion assignment and to quantify oxidized vs. unoxidized product ion abundances.

Top-down fragmentation of apomyoglobin was performed with an isolation window sufficiently wide to capture all detected oxidation products. A similar method used with oxidized calmodulin resulted in product ion spectra that were largely uninterpretable (unpublished results). Top-down fragmentation for calmodulin was performed using a narrow isolation window of 1 Da to yield more fragment coverage and reduce complexity in interpreting fragments.

**Bottom-up analysis**

For oxidized apomyoglobin and calmodulin, 50 mM of Tris pH 8.0 and 5 mM DTT were added to the protein, and the protein was heat denatured at 95°C for 30 min. After cooling to room temperature, samples were digested overnight with trypsin for apomyoglobin or GluC for calmodulin at 37°C at an enzyme:protein ratio of 1:20. After digestion, the enzyme was inactivated with 0.1% formic acid. Prior to injection, samples were incubated with 5 mM DTT at 65°C. The digested samples were stored in −20°C until LC-MS/MS analysis. One microliter of the digested sample was injected onto a PepMap reversed phase LC 75 µm × 15 cm C18 analytical column (2 µm particle size, 100Å pore size) using an Ultimate 3000 Nano LC system ( Dionex, Thermo Fisher Scientific) coupled to a Thermo Orbitrap Fusion Tribrid (Thermo Fisher Scientific) in positive mode with spray voltage 2300, capillary temperature 200°C at 60,000 nominal Orbitrap resolution. Mobile phase A was LC-MS grade water, 0.1% formic acid, and mobile phase B was ACN with 0.1% formic acid. A gradient elution was performed by equilibrating with 2% mobile phase B for 6 min, increasing to 35% mobile phase B over 23 min, further increasing to 95% over 5 min, holding at 95% mobile phase B for 3 min, returning to 2% mobile phase B over 10 min. Samples were run in technical triplicate for statistical purposes.

Data were processed using Thermo Xcalibur. The peptide peaks were extracted with a mass tolerance of 10 parts/million and quantified by peak area integration for both oxidized and unoxidized peaks. Sequence coverage and
MS/MS fragments were validated with Byonic v.2.10.5 software (Protein Metrics, Cupertino, CA, USA), with variable modification of methionine, 2 missed cleavages, and 10 parts per million mass tolerance. MS/MS fragments were validated manually to avoid false positives.

RESULTS AND DISCUSSION

Bottom-up quantification of Met oxidation in apomyoglobin

In order to test the accuracy of top-down measurements, we first measured methionine oxidation of our sample by standard bottom-up methods for comparison. The distance between the 2 methionines makes the measurement by bottom-up MS/MS relatively straightforward and reliable. Two partial digestion product peptides were identified containing M55, whereas only 1 peptide containing M131 was detected. Oxidized and unoxidized peaks from peroxide-free control was quantified, revealing 7% oxidation for M55 and 13% oxidation for M131. Intact MS analysis of myoglobin without peroxide treatment shows a very low amount of oxidation of the intact protein (Fig. 1A), indicating that most of this background oxidation occurred during digestion, LC-MS analysis, or both. This background oxidation was subtracted from the bottom-up data to calculate oxidation of each methionine. The corrected fraction of oxidation of each methionine after 6 h of peroxide incubation are shown in Fig. 1B. No oxidation of other peptides was detected at rates >1% (unpublished results). Annotated MS/MS spectra of the oxidized peptides are shown in Fig. 2. These spectra clearly show that oxidation occurred solely on the methionine within each peptide.

Top-down measurement of methionine oxidation in apomyoglobin

After incubation with hydrogen peroxide for 6 h and postoxidation clean-up, we infused the intact myoglobin for electrospray ionization–MS using propylene carbonate to increase charging and help ensure a uniform high charge state distribution for the denatured intact protein to improve ETD fragmentation efficiency. All charge states detected gave an oxidation product distribution that reflected no clear cooperativity between oxidation, with a distribution of M: M+O:M+2O of ~0.16:0.51:0.33 (Fig. 3). We selected the +23 charge state for top-down ETD analysis. Distribution of oxidation products of the +27 charge state looked identical to the +23 charge state (unpublished results). Top-down fragmentation was performed using a 10 m/z units window ensure that all oxidation states for a given charge state were isolated and fragmented. Postisolation intact MS showed no substantial change in the distribution of oxidation products, indicating the isolation window was wide enough to prevent losses (unpublished results).

Figure 4A shows the sequence coverage of the +23 charge state of apomyoglobin after incubation with peroxide for 6 h. We were able to achieve sequence coverage across both oxidized methionines: M55 with the c-ion series and M131 with the z-ion series. Representative zoomed-in, annotated ETD spectra for each product ion quantified are presented in Supplemental Fig. S1A–O. Product ions were screened for mass accuracy, presence in the unoxidized control sample, and proper charge state as determined by [13C] isotopic clusters. As represented by the C54 ion (Supplemental Fig. S1A), no oxidation was detected for c-ions prior to M55. Oxidation of M55 was measured using 2 nonadjacent c-ions identified in both technical replicates, as shown in Fig. 4B. The measurements based on either c-ion were very consistent with one another (0.60 ± 0.10 vs. 0.53 ± 0.03). Importantly, both measurements were close to the value that was obtained by bottom-up analysis, as shown in Fig. 4D. Moreover, the unoxidized control apomyoglobin showed no detectable levels of oxidized protein by intact MS analysis (unpublished results).
Figure 4C shows measurement of oxidation of M131 using the z-ion series. Because M131 was closer to the C terminus, we were able to measure a larger number of product ions after M131 using the top-down method. More variability is observed in the amount of oxidation measured from z-ions as compared with c-ions, whereas reproducibility between technical replicates remained high. No clear trends explaining the variability are immediately obvious based on analysis of which product ions overestimate vs. underestimate methionine oxidation. Comparison of the fragmentation pattern from the unoxidized control indicated that interference from misassignment of y-ions was not the cause of variability in quantification using the z-ion series (unpublished results). The variation in quantification from the z-ion series appears to center on the level of oxidation measured by bottom-up methods, suggesting that although an individual z-ion may overestimate or underestimate methionine oxidation, the mean of all oxidation measurements may be more accurate. As shown in Fig. 4D, the mean measured amount of oxidation for all oxidized z-ions reports an oxidation value for M131 of 0.39 ± 0.11 after 6 h of incubation, within measurement error of the value of 0.47 ± 0.03 obtained from traditional bottom-up methods.

**Charge state dependence of top-down quantification of methionine oxidation**

In order to test the charge state dependence of top-down quantitation of methionine oxidation, we generated a second sample after overnight oxidation that yielded a similar number of oxidation events per molecule based on intact ion intensity (~1.05 oxidations per molecule for the 6-h incubation period). Deconvoluted mass spectrum of intact protein apomyoglobin after peroxide incubation for 6 h (inset: +23 charge state). All detected charge states reflected a similar distribution of oxidation products.

**FIGURE 2**

ETD MS/MS spectra of oxidized peptides from bottom-up analysis, as annotated by Byonic and validated manually. A, B) Representative ETD spectrum of singly oxidized peptides containing M55. C) Representative ETD spectrum of singly oxidized peptide containing M131. In no case was oxidation of any amino acid other than the 2 methionines detected at a level of 1% or higher.

**FIGURE 3**

Deconvoluted mass spectrum of intact protein apomyoglobin after peroxide incubation for 6 h (inset: +23 charge state). All detected charge states reflected a similar distribution of oxidation products.
sample; ~1.17 oxidations per molecule for the 12-h incubation sample). We used this sample to perform top-down analysis of the +27 charge state. The results of this analysis are shown in Fig. 5. Performing ETD at different charge states altered the product ions that were observed; representative spectra of each detected product ion are shown in Supplemental Fig. S2A–P. However, the same general trends were observed for the +27 charge state ions as for the +23 charge state. The c-ion series gave more consistent oxidation values (Fig. 5B). The z-ion series gave more variable values, with no obvious patterns regarding which product ion underestimated oxidation and which overestimated (Fig. 5C). Many z-ions gave consistent results between charge states, but not all. Indeed, a z-ion could underestimate oxidation in the +27 charge state but overestimate oxidation in the +23 charge state (e.g., z25), or vice versa (e.g., z38). However, the overall trend for the mean z-ion oxidation measurements to reflect values obtained by bottom-up at this level of overall oxidation held (Fig. 5D). The +27 charge state gave a value for fractional oxidation of M55 of 0.53 ± 0.03 and a value for fractional oxidation of M131 of 0.38 ± 0.11. This level was consistent with that of the +23 charge state and was similar to that observed by bottom-up analysis (Fig. 4).

**Bottom-up quantification of Met oxidation in calmodulin**

Although apomyoglobin offers a simple model system, we tested the ability of ETD-based top-down MS/MS to accurately quantify methionine oxidation in a more complex system. Calmodulin has 9 methionines, and their distance from the N and C termini ensures that we have numerous measurements prior to the methionine to test the likelihood of misidentification of product ion oxidation. In order to test the accuracy of top-down measurements, we first measured methionine oxidation of calmodulin by standard bottom-up methods. All 9 methionines were detected in 6 different GluC peptides, and oxidation was quantified for each peptide (Fig. 6). MS/MS spectra reveal no clear evidence of oxidation of residues other than methionine, and no oxidation over 1% was detected in peptides that do not contain methionine (unpublished results).

**Top-down measurement of methionine oxidation in calmodulin**

After incubation with hydrogen peroxide for 1 h and postoxidation clean-up, we infused the intact calmodulin for electrospray ionization–MS, using ACN, formic acid, and propylene carbonate to increase charging and help ensure a uniform high charge state distribution for the denatured intact protein. All charge states detected gave an oxidation product distribution that reflected no clear cooperativity between oxidation (Fig. 7).

A wide isolation window that captured all oxidized versions of the +15 charge state followed by ETD gave poor
sequence coverage with no quantifiable oxidized product ions (unpublished results). In order to simplify the sample, we isolated and performed ETD on just the M+O oxidation product. Figure 8A shows the sequence coverage of calmodulin isolated M+O 1121.400 m/z after incubation with peroxide for 1 h. We were able to achieve good sequence coverage for oxidized methionine’s M76. Oxidation of c-ions, as measured based on oxidized vs. total product ion intensities, represents the mean oxidation of M76. Error bars indicate 1 SD (Fig. 8B). Representative zoomed-in, annotated ETD spectra for each product ion quantified in Fig. 8B are presented in Supplemental Fig. S3A–G. Figure 8C compares the mean of all values of oxidation for M76 obtained by bottom-up and top-down means. Product ions were screened for mass accuracy, presence in the unoxidized control sample, and proper charge state as determined by [13C] isotopic clusters. As represented by the c75 ion (Supplemental Fig. S3A), no oxidation was detected for c-ions prior to M76 even though several methionines N-terminal to residue K75 are detected as heavily oxidized in bottom-up data (Fig. 6). Oxidized ETD ions could only be detected in the c-ion series after M76, and the quantification of these ETD ions does not match with bottom-up data.

These results indicate that, for calmodulin, top-down ETD fails to properly identify all oxidized methionines. Because of the inability to obtain oxidized product ions for simultaneous ETD fragmentation of all oxidized versions of the +15 charge state, it is not straightforward to quantitatively compare top-down measurement of oxidation of M76 from the +16 Da precursor with bottom-up measurement of the total oxidation of the
peptide containing M76 (as well as M71 and M72) from all calmodulin oxidation products. However, unlike for apomyoglobin c-ions, calmodulin c-ions displayed a high variability in measured product ion oxidation. Several c-ions, including c75, were detected with good abundance but showed no oxidized product ion, despite M51 being the most readily oxidized amino acid in calmodulin.

**CONCLUSIONS**

Based on the data presented here, top-down ETD-based MS/MS is not able to consistently quantify oxidation of methionine oxidation, even under denaturing and supercharging conditions. Although top-down measurements of methionine oxidation in apomyoglobin were in good agreement with bottom-up measurements, the method failed to accurately quantify oxidation of methionine in a more complex calmodulin system. In most cases, calmodulin oxidation even failed to identify sites of oxidation. For example, bottom-up data clearly show M51 to be the most readily oxidized methionine in the protein (Fig. 6); however, numerous c-ion products were detected between M51 and K75, none of which exhibited a detectable oxidized version of the product ion (Fig. 8A). Application of multiple deconvolution algorithms failed to detect these oxidized product ions (unpublished results). Although previous work clearly demonstrated that methionine oxidation (along with other amino acid oxidation products) could be clearly detected and quantified by ETD MS/MS,19, 25, 26 this method is currently unreliable for top-down analysis. The success of this approach in the simpler apomyoglobin system may indicate that the failure is due to the complexity of the calmodulin system rather than an inherent property of ETD.

**FIGURE 7**

Deconvoluted mass spectrum of the intact protein calmodulin after peroxide incubation for 1 hr (inset: +15 charge state). All detected charge states reflected a similar distribution of oxidation products.

**FIGURE 8**

ETD-based top-down analysis of all oxidized and unoxidized forms of the +15 charge state of calmodulin after peroxide oxidation for 1 hr. A) Sequence coverage of calmodulin. Ions labeled in red were only detected as unoxidized. Ions labeled in green were detected in both oxidized and unoxidized forms and were used for oxidation quantification. Ions labeled in black were undetected or had insufficient intensity for quantification. B) Oxidation of c-ions, as measured based on oxidized vs. total product ion intensities. Red hashed line represents the mean total oxidation of M71, M72, and M76 based on bottom-up data; error bars indicate 1 SD. C) Mean of all values of oxidation taken for M76 from top-down analysis and bottom-up analysis.
fragmentation. Further experiments using high-field Fourier transform–ion cyclotron resonance, as well as application of electron capture dissociation, may enable more robust top-down methionine oxidation.

ACKNOWLEDGMENTS

The authors thank Dr. Sandeep Misra for assistance. This material is based on work supported by the National Science Foundation under Grant 1608685. J.S.S. discloses a significant financial interest in GenNext Technologies, Inc., a small company seeking to commercialize technologies for protein higher order structure analysis.

REFERENCES

1. Dean RT, Fu S, Stocker R, Davies MJ. Biochemistry and pathology of radical-mediated protein oxidation. Biochem J. 1997;324:1–18.
2. Hoshi T, Heinemann S. Regulation of cell function by oxidation and reduction. J Physiol. 2001;531:1–11.
3. Levine RL, Mosoni L, Berlett BS, Stadtman ER. Methionine residues as endogenous antioxidants in proteins. Proc Natl Acad Sci USA. 1996;93:15036–15040.
4. Jansen-Heininger YMW, Mossman BT, Heintz NH, et al. Redox-based regulation of signal transduction: principles, pitfalls, and promises. Free Radic Biol Med. 2008;45:1–17.
5. Berlett BS, Stadtman ER. Protein oxidation in aging, disease, and oxidative stress. J Biol Chem. 1997;272:20313–20316.
6. Gao J, Yin DH, Yao Y, et al. Loss of conformational stability in calmodulin upon methionine oxidation. Biophys J. 1998;74:1115–1134.
7. Ciorba MA, Heinemann SH, Weissbach H, Brot N, Hoshi T. Modulation of potassium channel function by methionine oxidation and reduction. Proc Natl Acad Sci USA. 1997;94:9932–9937.
8. Palmblad M, Westlind-Danielsson A, Bergquist J. Oxidation of methionine 35 attenuates formation of amyloid β-peptide 1-40 oligomers. J Biol Chem. 2002;277:19506–19510.
9. Schmalstig AA, Benoit SL, Misra SK, Sharp JS, Maier RJ. Noncatalytic antioxidant role for Helicobacter pylori urease. J Bacteriol. 2018;200:e00124-18.
10. Arnér ES, Holmgren A. Physiological functions of thioredoxin and thioredoxin reductase. Eur J Biochem. 2000;267:6102–6109.
11. Leader B, Baca QJ, Golan DE. Protein therapeutics: a summary and pharmacological classification. Nat Rev Drug Discov. 2008;7:21–39.
12. Wang W. Instability, stabilization, and formulation of liquid protein pharmaceuticals. Int J Pharm. 1999;185:129–188.
13. Kerwin BA, Remmele RL Jr. Protect from light: photodegradation and protein biologics. J Pharm Sci. 2007;96:1468–1479.
14. Torosantucci R, Mozziconacci O, Sharov V, Schöneich C, Jiskoot W. Chemical modifications in aggregates of recombinant human insulin induced by metal-catalyzed oxidation: covalent cross-linking via michael addition to tyrosine oxidation products. Pharm Res. 2012;29:2276–2293.
15. Nguyen TH. Oxidation degradation of protein pharmaceuticals. In Formulation and Delivery of Proteins and Peptides. Washington, DC: American Chemical Society, 1994:59–71.
16. Li S, Schöneich C, Borchardt RT. Chemical instability of protein pharmaceuticals: mechanisms of oxidation and strategies for stabilization. Biotechnol Bioeng. 1995;48:490–500.
17. Sharp JS, Tomer KB. Analysis of the oxidative damage-induced conformational changes of apo- and holocalmodulin by dose-dependent protein oxidative surface mapping. Biophys J. 2007;92:1682–1692.
18. Li X, Li Z, Xie B, Sharp JS. Improved identification and relative quantification of sites of peptide and protein oxidation for hydroxyl radical footprinting. J Am Soc Mass Spectrom. 2013;24:1767–1776.
19. Li X, Li Z, Xie B, Sharp JS. Supercharging by m-NBA improves ETD-based quantification of hydroxyl radical protein footprinting. J Am Soc Mass Spectrom. 2015;26:1424–1427.
20. Riley NM, Coon JJ. The role of electron transfer dissociation in modern proteomics. Anal Chem. 2018;90:40–64.
21. Jiang X, Smith JB, Abraham EC. Identification of a MS-MS fragment diagnostic for methionine sulfoxide. J Mass Spectrom. 1996;31:1309–1310.
22. Reid GE, Roberts KD, Kapp EA, Simpson RI. Statistical and mechanistic approaches to understanding the gas-phase fragmentation behavior of methionine sulfoxide containing peptides. J Proteome Res. 2004;3:751–759.
23. Ogorzalek Loo RR, Lakshmanan R, Loo JA. What protein charging (and supercharging) reveal about the mechanism of electrospray ionization. J Am Soc Mass Spectrom. 2014;25:1675–1693.
24. Fellers RT, Greer JB, Early BP, et al. ProSight lite: graphical software to analyze top-down mass spectrometry data. Proteomics. 2015;15:1235–1238.
25. Xie B, Sharp JS. Relative quantification of sites of peptide and protein modification using size exclusion chromatography coupled with electron transfer dissociation. J Am Soc Mass Spectrom. 2016;27:1322–1327.
26. Srikanth R, Wilson J, Bridgewater JD, et al. Improved sequencing of oxidized cysteine and methionine containing peptides using electron transfer dissociation. J Am Soc Mass Spectrom. 2007;18:1499–1506.