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Investigation of the Mechanism of Electron Capture and Electron Transfer Dissociation of Peptides with a Covalently Attached Free Radical Hydrogen Atom Scavenger

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Abstract

The mechanisms of electron capture and electron transfer dissociation (ECD and ETD) are investigated by covalently attaching a free-radical hydrogen atom scavenger to a peptide. The 2,2,6,6-tetramethylpiperidin-l-oxyl (TEMPO) radical was chosen as the scavenger due to its high hydrogen atom affinity (ca. 280 kJ/mol) and low electron affinity (ca. 0.45 ev), and was derivatized to the model peptide, FQX^{TEMPO}EEQQQTEDELQDK. The X^{TEMPO} residue represents a cysteinyl residue derivatized with an acetamido-TEMPO group. The acetamide group without TEMPO was also examined as a control. The gas phase proton affinity (882 kJ/mol) of TEMPO is similar to backbone amide carbonyls (889 kJ/mol), minimizing perturbation to internal solvation and sites of protonation of the derivatized peptides. Collision induced dissociation (CID) of the TEMPO tagged peptide dication generated stable odd-electron b and y type ions without indication of any TEMPO radical induced fragmentation initiated by hydrogen abstraction. The type and abundance of fragment ions observed in the CID spectra of the TEMPO and acetamide tagged peptides are very similar. However, ECD of the TEMPO labeled peptide dication yielded no backbone cleavage. We propose that a labile hydrogen atom in the charge reduced radical ions is scavenged by the TEMPO radical moiety, resulting in inhibition of N−C_α backbone cleavage processes. Supplemental activation after electron attachment (ETcaD) and CID of the charge-reduced precursor ion generated by electron transfer of the TEMPO tagged peptide dication produced a series of b + H (b^H) and y + H (y^H) ions along with some c ions having suppressed intensities, consistent with stable O−H bond formation at the TEMPO group. In summary, the results indicate that ECD and ETD backbone cleavage processes are inhibited by scavenging of a labile hydrogen atom by the localized TEMPO radical moiety. This observation supports the conjecture that ECD and ETD processes involve long-lived intermediates formed by electron

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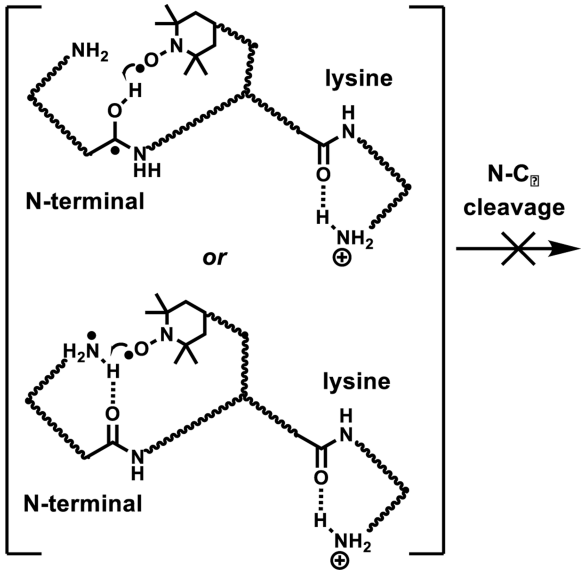
capture/transfer in which a labile hydrogen atom is present and plays a key role with low energy processes leading to c and z ion formation. Ab initio and density functional calculations are performed to support our conclusion, which depends most importantly on the proton affinity, electron affinity and hydrogen atom affinity of the TEMPO moiety.

Graphical Abstract



Keywords

TEMPO; Electron Capture Dissociation; Electron Transfer Dissociation; Free Radical; Peptide; Reaction Mechanism

1. Introduction

Electron capture dissociation (ECD) [1] and electron transfer dissociation (ETD) [2] are invaluable tools for elucidating structures of peptides and proteins in mass spectrometry (MS)-based proteomics. Applications include peptide sequencing [3, 4], identification of post-translational modifications [5–7] and studies of non-covalent protein complexes [8–10]. Thermal ion activation methods such as collision induced dissociation (CID) [11–13] and infrared multi-photon dissociation (IRMPD) [14, 15] accumulate vibrational excitation into peptide or protein ions, mainly leading to rupture of C−N amide bonds to produce b and y type ions (Scheme 1). In contrast, ECD involves electron capture by multiply charged peptides and proteins to form radical cations that mainly undergo N−C_α bond cleavage, generating unique c and z type fragments [16, 17] (Scheme 1).

For more than a decade, experimental and theoretical studies have attempted to illuminate the mechanism of ECD and ETD [18, 19]. Factors influencing the prominent N−C_α bond cleavage processes leading to c and z ions in ECD have been a major focus of these investigations. These wide ranging investigations include tagging peptides with permanent charged groups [20–27], electron/hydrogen atom scavengers [28–33] and modified peptide

backbones [34–38]. These experiments are primarily designed to monitor the initial electron capture and subsequent electronic state relaxation via internal conversion and intramolecular electron, proton and hydrogen atom transfer processes. Mobility of an electron or hydrogen atom formed by initial electron capture appears to play a critical role in ECD backbone fragmentation pathways [39]. However, even without a mobile electron or hydrogen atom, ECD backbone fragmentation processes are still observed [40], possibly implicating the involvement of electronically excited state-mediated processes [17].

The effect of hydrogen atom scavengers in ECD was previously studied by the O'Connor group by conjugating the even-electron coumarin group (Figure 1a) to peptide Substance P [28]. Termination of amide backbone fragmentation accompanied with abundant side-chain losses was observed. This was attributed to hydrogen radical scavenging by the coumarin group. However, starting with the even electron coumarin scavenger, hydrogen scavenging yields a free radical product, that might be involved in subsequent interactions with the covalently attached peptide residues. Details in the energetics and mechanism of hydrogen atom scavenging by the coumarin group were not fully elucidated.

An independent investigation on the operation of a different type of radical scavenger, 2-(4'-carboxypyrid-2'-yl)-4-carboxamide (pepy) group, has been performed by the Turecek group [29]. The pepy group has a higher gas phase basicity (923 kJ/mol) than the amide backbones in their model peptides, and thus is likely protonated in doubly and triply charged ions. The recombination energy (5.43-5.46 eV) and hydrogen atom affinity (~160 kJ/mol) of the pepy residue are also higher than all other charged sites in the bioconjugates, which eventually traps an electron at the pepy residue acting as permanently charged tags. The initially captured electron, which is immobilized at the pepy residue, does not trigger typical radical driven ECD backbone fragmentations, producing C−N amide bond fragments through vibrational excitation acquired by electron capture and additional infra-red photon irradiation. However, the initial protonation sites of peptides are disrupted by labeling the pepy group, which also changes the overall electron capture and subsequent fragmentation pathways.

As seen in previous studies [28–33], it is clear that ECD backbone fragmentation processes can be inhibited by scavenging labile hydrogen atoms produced by electron capture. In this study, we take a different approach than those used in the previous studies to investigate reaction dynamics of the nascent charge-reduced peptide ions after initial electron capture and subsequent electronic relaxation. For this study, a well-defined radical group is conjugated to the model peptide, with the ability to abstract a labile hydrogen atom either from a hypervalent amine or an aminoketyl radical before dissociation can occur following electron capture. This chemical moiety should exhibit minimal disruption of the initial electron capture process. To satisfy the requirements above, we chose the covalently attached free-radical hydrogen atom scavenger incorporating the 2,2,6,6-tetramethylpiperidin-l-oxyl (TEMPO) group to investigate the mechanism of electron capture and electron transfer dissociation (Figure 2). Of particular interest is that the TEMPO group has the fairly similar proton affinity (882 kJ/mol) [41] to that of the amide backbone (887 kJ/mol), ensuring less perturbation of the site of protonation and the pattern

of internal solvation. Finally, the electron affinity of the TEMPO group is ca. 0.45 eV, which is low enough not to hinder ECD backbone fragmentation by trapping an electron [30].

With these considerations, we investigated the efficacy of the TEMPO group as a covalently attached free-radical hydrogen atom scavenger in fragmentation of multiply charged peptides triggered by electron attachment. We observe the inhibition of typical c and z type ion formation in ECD and ETD of the TEMPO labeled peptide. High level *ab initio* and density functional theory (DFT) calculations are performed to estimate the proton and hydrogen atom affinities of the TEMPO group. These results provide clear clues regarding the energetics and dynamics of electron attachment triggered fragmentation processes of the multiply charged peptides with and without the TEMPO label.

2. Experimental

2.1 Materials

A monophosphopeptide from β-casein (FQpSEEQQQTEDELQDK) was purchased from Anaspec (San Jose, CA). Iodoacetamide, 4-(2-iodoacetamide)-TEMPO, 1,2-ethanedithiol and 0.3 N $Ba(OH)_2$ were provided by Sigma-Aldrich. All solvents were obtained from EMD Merck. The 100 μL size C18 desalting tip was acquired from Varian Inc.

2.2 Sample Preparation

A 20 μg portion of the model peptide, FQpSEEQQQTEDELQDK, was dissolved in 40 μL of a 4:3:1 mixture of H_2O, ethanol and DMSO. The phosphoserine residue was converted to dehydroalanine (dA) under basic solution conditions by adding 20 μL of 0.3 N $Ba(OH)_2$ and incubating for 1 hr at room temperature. A 1 μL portion of 1,2-ethanedithiol was subsequently added to the reaction mixture for Michael addition and reacted for 3 hr at 37 °C, yielding an ethanethiolcysteine at the dehydroalaine residue. After the completion of the reaction, the solvent was completely removed by Speed-vac and the residue was reconstituted to 100 μL with 0.1 % formic acid for desalting. After desalting using the C18 tips, the solvent was exchanged to 100 mM Na_2HPO_4 buffer (pH~8.5). It should be noted that successive oxidation of the ethanethiolcysteine residue with formation of multiple disulfide bond containing residues was observed. A series of resulting peptide peaks appeared in the MALDI-TOF mass spectrum showing sequential +92 Da increases that corresponds to the mass of [S-CH_2CH_2-S]. A free thiol group was regenerated by adding 1.5 μL of 100 mM dithiothreitol and incubating for 15 min at 37 °C. The reaction mixture was reacted with 3 μL of 100 mM 4-(2-Iodoacetamido)-TEMPO in acetonitrile for 1 hr at room temperature in the dark, yielding the final TEMPO-derivatized peptide. The solvent was removed by Speed-vac and the residue was subject to the desalting step. The TEMPO-labeled peptide (**1**, Figure 2) was eluted to 100 μL with 0.1 % formic acid and 50% acetonitrile and directly infused to the mass spectrometers using a nanospray ion source. Iodoacetamide (IAA) was also derivatized to the model peptide (**2**, Figure 2) using the same reaction condition for control experiments.

2.3 Mass Spectrometry

A 7 Tesla linear ion trap Fourier transform ion cyclotron resonance (LTQ-FTICR) mass spectrometer (Thermo Scientific, San Jose, CA) was used for CID and ECD experiments. Desalted samples after conjugation reactions were further diluted by 0.1% formic acid, 50% acetonitrile solvent by 20 fold and directly infused to the mass spectrometers using a nanospray ionization source with borosilicate emitters (Proxeon, Cambridge, MA). The spraying voltage was set to 1.2-1.5 kV. Critical instrumental parameters were a capillary temperature of 200 °C, a capillary voltage of 36-49 V, and a tube lens offset of 200 V. Other ion optics parameters were optimized for maximum intensities of ions. Electron irradiation time for ECD experiments was 100 ms at 5% of the full energy scale (<1 eV, nearly thermal electron). CID spectra were recorded at the collision energy of 15 %. The target resolution was 100,000 fwhm and 100 scans were averaged for ECD and CID spectra.

A linear ion trap-orbitrap (LTQ-orbitrap) mass spectrometer (Thermo Scientific) was used for ETD and supplemental activation experiments. The same parameters for nanospray and ion optics were used as for FTICR. Fluoranthene was used for ETD and related experiments as a negative ion reagent. Critical parameters for the ETD reagent were an emission current of 110 μA, a CI gas pressure of 25 psi, and a source temperature of 200 °C. Other ion optics parameters for transfer of the fluoranthene anion were optimized using the auto-tune method and the signal level of the fluoranthene anion at m/z 202 was ~2.5e6 and stayed stable during the course of ETD experiments. The activation time, roughly corresponding to the ion/ion reaction time, of the ETD experiments was varied from 50 to 200 ms by monitoring intensities of fragment ions. Supplemental activation after electron transfer for ETcaD experiments was performed at 10% collision energy targeted to isolated 2+ ions. Further CID of the charge-reduced precursor radical cation was performed by isolation of the target ions, followed by collisional activation at 15% energy. The target resolution was 60,000 fwhm and 100 scans were averaged for ETD and related experiments.

2.4 Calculations

All density functional and *ab initio* calculations were performed using Gaussian 03 (Revision E.01) for evaluation of electron affinity, proton affinity and hydrogen affinity of TEMPO. The initial geometry of TEMPO radical (TEMPO$^{\bullet}$), TEMPO anion (TEMPO^{-}), protonated TEMPO radical (TEMPO-H$^{+\bullet}$), and hydrogen atom scavenged TEMPO (TEMPO-H) were optimized using Becke's hybrid functional (B3LYP) [42, 43] with the 6-31+G(d,p) basis set. For odd-electron systems, the spin-unrestricted formalism (UB3LYP) was used. Spin contamination of UB3LYP calculations was very low ($<S^2> \sim 0.754$), validating usage of the spin-unrestricted method. Thermochemical properties of the model systems were obtained by vibrational frequency calculations at the same level of theory at 298.15 K and 1.0 atm. Local minima were confirmed by inspecting the number of imaginary vibrational frequencies. Refined electronic energies were acquired from using B3LYP, BMK [44], M05-2X [45, 46] density functionals and the second-order Møller-Plesset perturbation theory (MP2) with the 6-311++(3df,2pd) basis set as implemented in Gaussian 03 suite of programs. Simple average of B3LYP and MP2 (B3-MP2) was also obtained as described previously for comparison [47]. Spin-projected MP2 (PMP2) energy and its average with B3LYP (B3-PMP2) were also considered.

3. Results and discussion

Table 1 summarizes the electron affinity, proton affinity, and hydrogen atom affinity of TEMPO calculated by various methods. All methods estimate the proton affinity to be reasonably close to the experimental value (882 kJ/mol), ranging from 873.0 to 893.1 kJ/mol. B3-MP2 and B3-PMP2 give the most accurate values, differing with the experimental value less than 2 kJ/mol as reported previously [47]. It should be noted that the proton affinity of the TEMPO residue is very close to that of the amide backbone ($CH_3CONHCH_3$, 888.5 kJ/mol) [41]. Since protonation of peptides occurs on basic residues, the sites of protonation in peptide dications will likely not be affected by attachment of the TEMPO group. Thus it minimizes structural changes in the bioconjugates relative to the underivatized peptide. Electron affinities predicted by various methods have a wide range from 0.296 (BMK) to 0.644 eV (MP2). We choose the value of the electron affinity of TEMPO as ~0.45 eV for our discussion by taking an approximate median of different methods. Hydrogen atom affinities of TEMPO predicted by BMK, M05-2X, B3-MP2, and B3-PMP2 are in the range of ~280 kJ/mol, which is higher than those of the pepy (~160 kJ/mol) and the diazirine (142-211 kJ/mol) groups used in previous studies [29, 32].

We next investigated the role of the TEMPO group in electron attachment triggered dissociation methods by performing CID, ECD, ETcaD of $[\mathbf{1} + 2H]^{2+\bullet}$, and $[\mathbf{2} + 2H]^{2+}$ and CID of the charge-reduced cations generated by electron transfer. Figure 3a depicts CID of $[\mathbf{1} + 2H]^{2+\bullet}$, showing abundant b and y type ions. Note that $b_3^{\bullet}$-$b_{15}^{\bullet}$ and $y_{14}^{\bullet}$ are odd-electron radical ions containing a stable TEMPO radical group. No free radical induced fragmentation is observed by abstraction of C_α or C_β hydrogens, followed by side-chain loss or backbone cleavage via β-scission [48]. Likewise, CID of $[\mathbf{2} + 2H]^{2+}$ (Figure 3b) exhibits an almost identical fragmentation pattern by forming abundant b and y type ions. Note that all of these fragments are even-electron species. Based on our quantum mechanical calculations (Table 1) and previous reports, it is clear that the TEMPO residue is less reactive (hydrogen atom affinity = 280 kJ/mol) than other carbon-centered radicals (e.g. acetyl radical, 420 kJ/mol) that were previously used for peptide fragmentation [48]. Therefore, the TEMPO residue cannot initiate free radical induced fragmentation by abstraction of hydrogen atoms in the peptides studied. Lack of free radical induced fragmentation by the covalently attached TEMPO group in the CID spectra has been reported in previous studies [49, 50].

ECD of the TEMPO tagged $[\mathbf{1} + 2H]^{2+\bullet}$ (Figure 4a) exhibits essentially no N–C_α cleavage except for a w_{14} product ion. ECD of the acetamide tagged $[\mathbf{2} + 2H]^{2+}$ still generates c and z type ions. Introduction of the hydrogen atom scavenger, the TEMPO residue, interrupts backbone fragmentations that are normally observed in ECD of multiply charged peptide cations. According to generally accepted ECD mechanisms, the initial electron capture by multiply charged peptide cations occurs in high-lying Rydberg states even in the presence of the electron affinity tuned tags and, following relaxation process, dissipates recombination energies via intramolecular electron transfer to other low-lying states and internal conversion [17, 30]. Our previous study has shown that the tags having electron affinity greater than 1.0 eV with a strong cationic hydrogen bond can interfere with electron relaxation processes after the initial electron capture by trapping an electron at the tag [30]. Thus it is natural to

inquire whether the TEMPO residue can act as an electron trap. The electron affinity of the TEMPO group is approximately 0.45 eV (Table 1), significantly lower than 1.0 eV. In addition, no significant hydrogen bonding interactions are expected between protonated basic residues and the TEMPO group. Therefore, the TEMPO residue would not disturb either the initial electron capture or subsequent relaxation processes as an electron trap. As another significant factor, the same model peptide from the previous electron predator work [30] was used to minimize changes in the gas phase conformation and reaction dynamics. The chemical linkage of the tag for labeling is longer than that of the previous work [30], and contains more functional groups (two thioethers and one amide bond). However, it was also previously demonstrated that the connectivity of the electron affinity tuned tags to the peptide is independent to their role unless the linker has any electron withdrawing group [30]. With these observations and considerations, we conclude that the TEMPO radical interacts with the hypervalent radical site resulting from electron capture, abstracting a hydrogen atom to form a stable O−H bond. This process ultimately impedes the normal sequence of events leading to peptide bond dissociation triggered by electron attachment.

Further collisional activation after electron transfer was performed to investigate fragmentation pathways at higher levels of internal excitation. Supplemental activation after electron transfer (ETcaD) was performed as implemented in the LTQ-tune program [51]. Figure 5 depicts the ETcaD spectra of the TEMPO tagged [**1** + 2H]$^{2+\bullet}$ (Figure 5a) and the acetamide tagged [**2** + 2H]$^{2+}$ (Figure 5b), respectively. Most strikingly, abundant z ions observed in ETcaD of the acetamide tagged [**2** + 2H]$^{2+}$ (Figure 5b) are suppressed in ETcaD of the TEMPO tagged [**1** + 2H]$^{2+\bullet}$ (Figure 5a). A series of b + H (b^H) and y + H (y^H) ions are observed in Figure 5a. It indicates facile abstraction of a labile hydrogen atom from a hypervalent amine or an aminoketyl intermediate radical, terminating the formation of z ions. Slightly suppressed, but still existing c ions in ETcaD of the TEMPO tagged [**1** + 2H]$^{2+\bullet}$ are observed in Figure 5a. Note that inhibition of the z ion formation is observed mostly in proximity of the TEMPO residue. This result implies that hydrogen atom scavenging occurs by the direct contact with the TEMPO residue before N−C_α bond cleavage. The c_{14}^H and c_{15}^H in ETcaD of the TEMPO tagged [**1** + 2H]$^{2+\bullet}$ (Figure 5a) are probably formed by abstracting a hydrogen atom from the neutral radical z fragment by the TEMPO residue after ETD type cleavage. Without the TEMPO residue, initial electron capture and following relaxation processes lead to the typical formation of c and z ions observed in ETcaD of the acetamide tagged [**2** + 2H]$^{2+}$ (Figure 5b).

Additional experiments were performed in which the charge-reduced cations generated by electron transfer were isolated and further collisionally excited, ensuring additional vibrational excitation involving a longer time scale and targeted m/z activation for fragmentation (Figure 6). The overall patterns found in Figure 5 and Figure 6 are similar. Figure 6a depicts CID of the charge-reduced TEMPO labeled peptide cation, [**1** + 2H]$^+$. The type and intensity of fragment ions in Figure 6a are very similar to those observed in Figure 5a. More neutral losses from b and y ions are observed. Figure 6b depicts CID of the charge-reduced TEMPO labeled peptide cation, [**2** + 2H]$^{+\bullet}$. A series of z ions (z_7 to z_{13}) are observed in Figure 6b but many of the b and y ions, and neutral losses that are abundant in Figure 5b are suppressed or missing (Figure 6a). Again, a hydrogen atom is scavenged by

the TEMPO residue even over the longer time scale and electron attachment triggered fragmentation is inhibited in the presence of the hydrogen atom scavenger.

The fragmentation pathways observed in this work are schematically summarized in Figure 7. The initial electron transfer and subsequent recombination energy relaxation occur in ETcaD of the TEMPO labeled peptide dication. The TEMPO residue abstracts a hydrogen atom from proximate residues, generating a stable O−H bond (Figure 7a). The internal energy gained by electron transfer and supplemental activation will be redistributed to vibrational modes and induce amide backbone C−N bond cleavages. The proposed mechanism is supported by observation of b^H and y^H ions (Figure 5a and Figure 6a). Figure 7b describes the case where the TEMPO residue of the low internal energy precursor ion is distant to the solvated amide carbonyls in the vicinity of the protonated lysine side-chain. In this conformation, electron transfer to lysine side-chain is less likely to be interrupted by the TEMPO residue, ultimately leading to N−C_α bond cleavage generating c ions and z type neutral fragments (Figure 5b and Figure 6b).

Based on the observed results, we offer the following conclusions. First, long-lived intermediates during ECD and ETD fragmentation processes are present. The intermediates could be either an aminoketyl radical or a hypervalent amine. Second, because the N−C_α bond cleavage processes in ECD/ETD of TEMO-derivatized peptides occurs in competition with hydrogen atom scavenging of the TEMPO residue, the N−C_α bond cleavage proceeds after intramolecular vibrational redistribution (IVR) in the current model system. Finally, the termination of typical ECD/ETD backbone fragmentation can be effected by adding either high electron affinity tags or hydrogen atom scavengers such as the TEMPO group. Yet, the actual mechanisms of inhibition for each case are different, involving interruption of electronic relaxation processes in the case of high electron affinity tags and scavenging of a hydrogen atom after electronic relaxation for the TEMPO group, respectively.

4. Conclusion

We report electron capture/transfer dissociations of peptide dications derivatized with a highly localized radical moiety, TEMPO, which possesses a high hydrogen atom affinity and a low electron affinity. The TEMPO substituent does not abstract C_α or C_β hydrogen atoms under collisional activation, producing only b and y ions and some of these ions contain stable odd-electron species. However, the TEMPO residue is effective at scavenging a labile hydrogen atom formed by electron capture or transfer, concomitant with interruption of N−C_α bond cleavage. With supplemental collisional activation after electron transfer and formation of the stable O−H bond at the TEMPO residue, observed fragments result from typical even electron peptide amide bond cleavages, producing b + H and y + H ions. By contrast, a control model peptide lacking the TEMPO residue exhibits typical N−C_α bond cleavages, yielding abundant c and z ions. From these observations, we conclude that long-lived intermediates such as an aminoketyl radical and a hypervalent amine are present in ECD and ETD processes, in which a labile hydrogen atom can be abstracted by the TEMPO residue in competition with N−C_α bond cleavages. These results also support the conclusion that intermediates involved in ECD and ETD type peptide backbone fragmentations are relatively long lived.

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Highlights

- TEMPO radical has high hydrogen affinity (~280 kJ/mol) and low electron affinity (~0.45 eV).
- In ExD, H-atom scavenging by TEMPO radical interrupts $N-C_{\alpha}$ bond cleavage.
- Long-lived intermediates in ExD are implicated to accommodate H-atom scavenging.

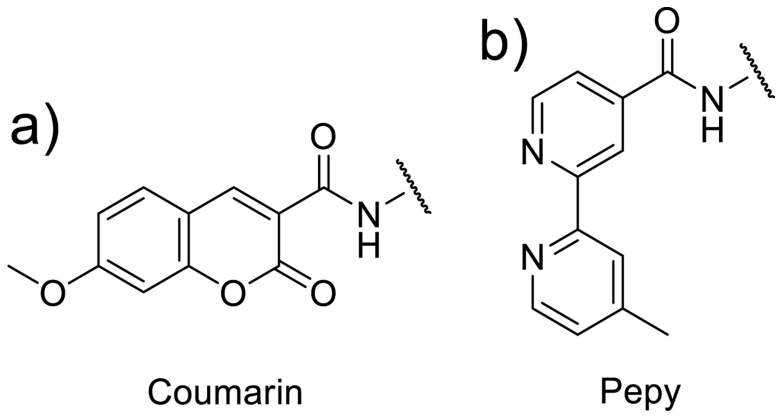


Figure 1.

Structures of a) coumarin and b) pepy groups used for the spin traps by O'Connor and Turecek groups [28, 29]. Both functional groups are conjugated to the N-terminal or the lysine ε-amine of the model peptides via amide coupling chemistry employing N-hydroxysuccinimine (NHS) activated reagents.

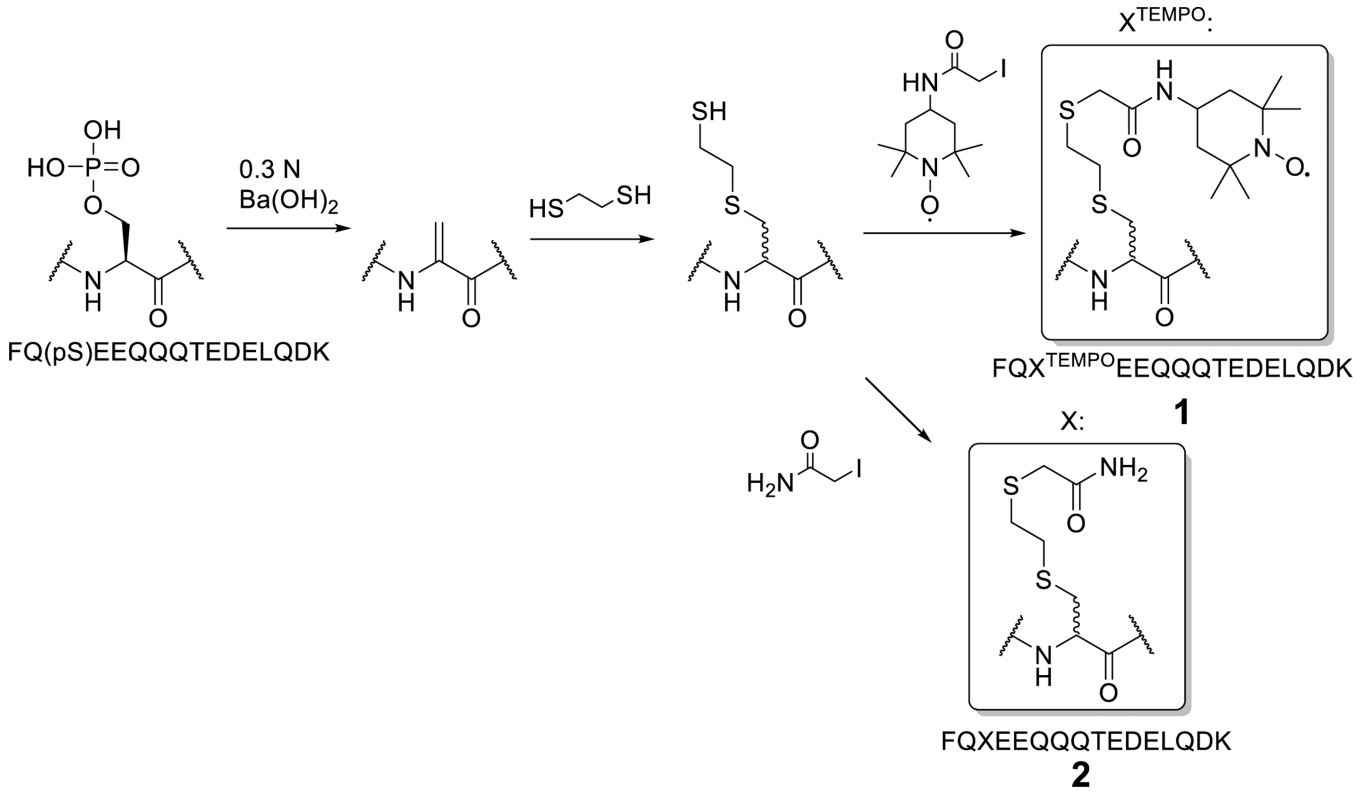


Figure 2.
Synthesis of TEMPO and acetamide-derivatized Peptide

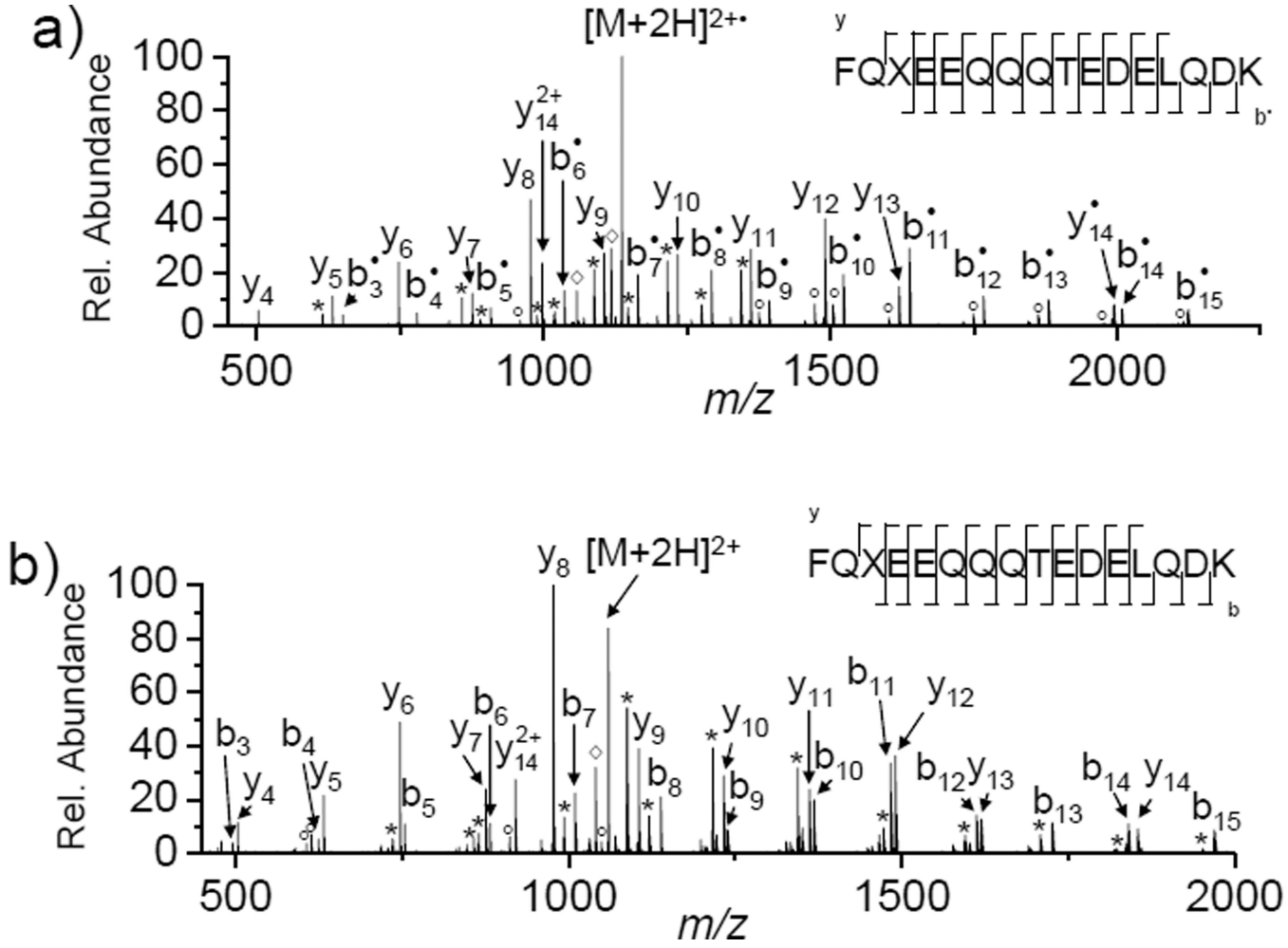


Figure 3.

CID of TEMPO and acetamide tagged peptide dications ([**1** + 2H]$^{2+\bullet}$ and [**2** + 2H]$^{2+}$), FQXEEQQQTEDELQDK, where X is TEMPO (**1**) or acetamide derivatized amino acid (2). a) CID of [**1** + 2H]$^{2+\bullet}$, and b) CID of [**2** + 2H]$^{2+}$. *, ° and ◊ indicate loss of NH_3, H_2O and (NH_3 + H_2O), respectively.

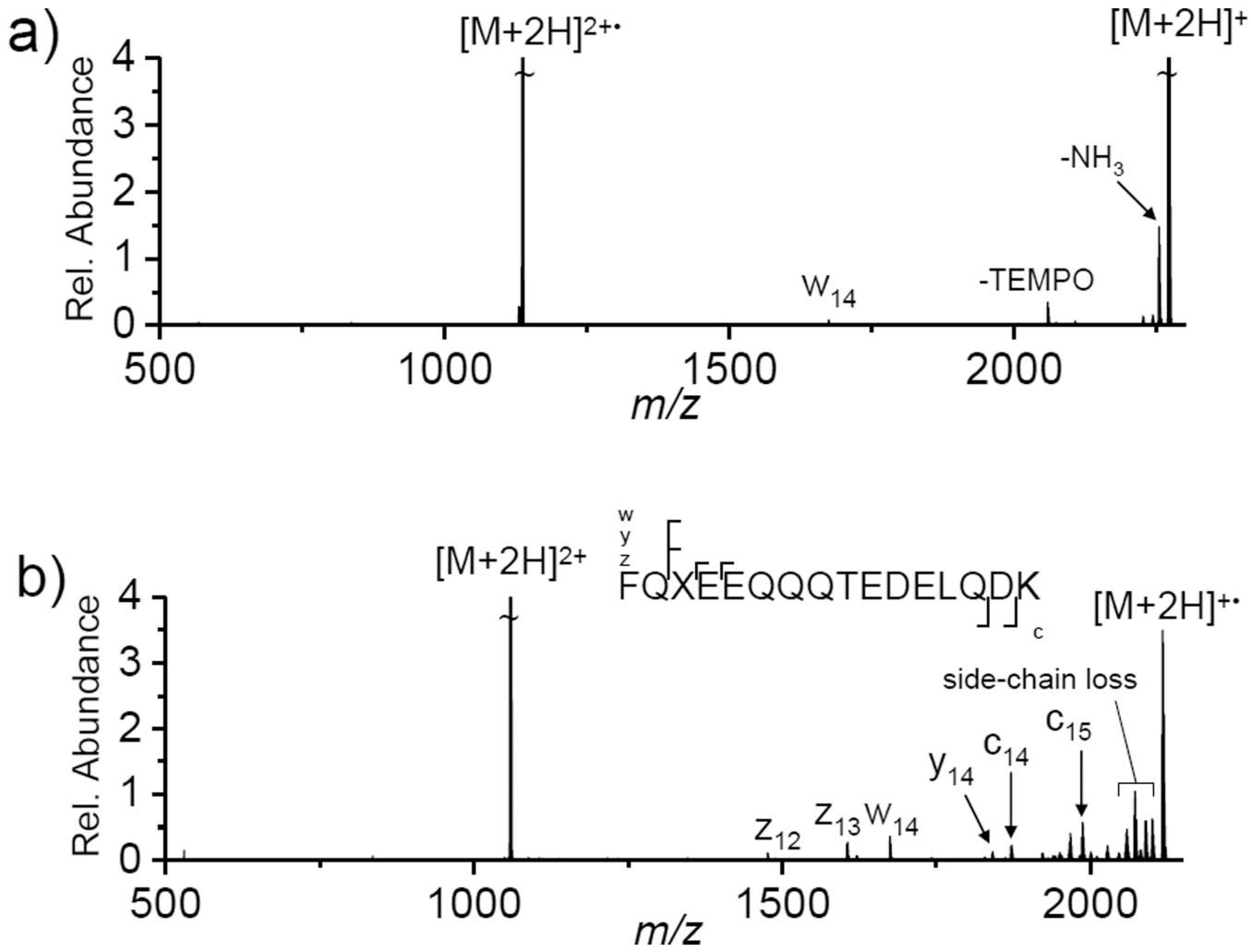


Figure 4.

ECD of TEMPO and acetamide tagged peptide dications ([**1** + 2H]$^{2+\bullet}$ and [**2** + 2H]$^{2+}$), FQXEEQQQTEDELQDK, where X is TEMPO (**1**) or acetamide derivatized amino acid (**2**). a) ECD of [**1** + 2H]$^{2+\bullet}$, and b) ECD of [**2** + 2H]$^{2+}$.

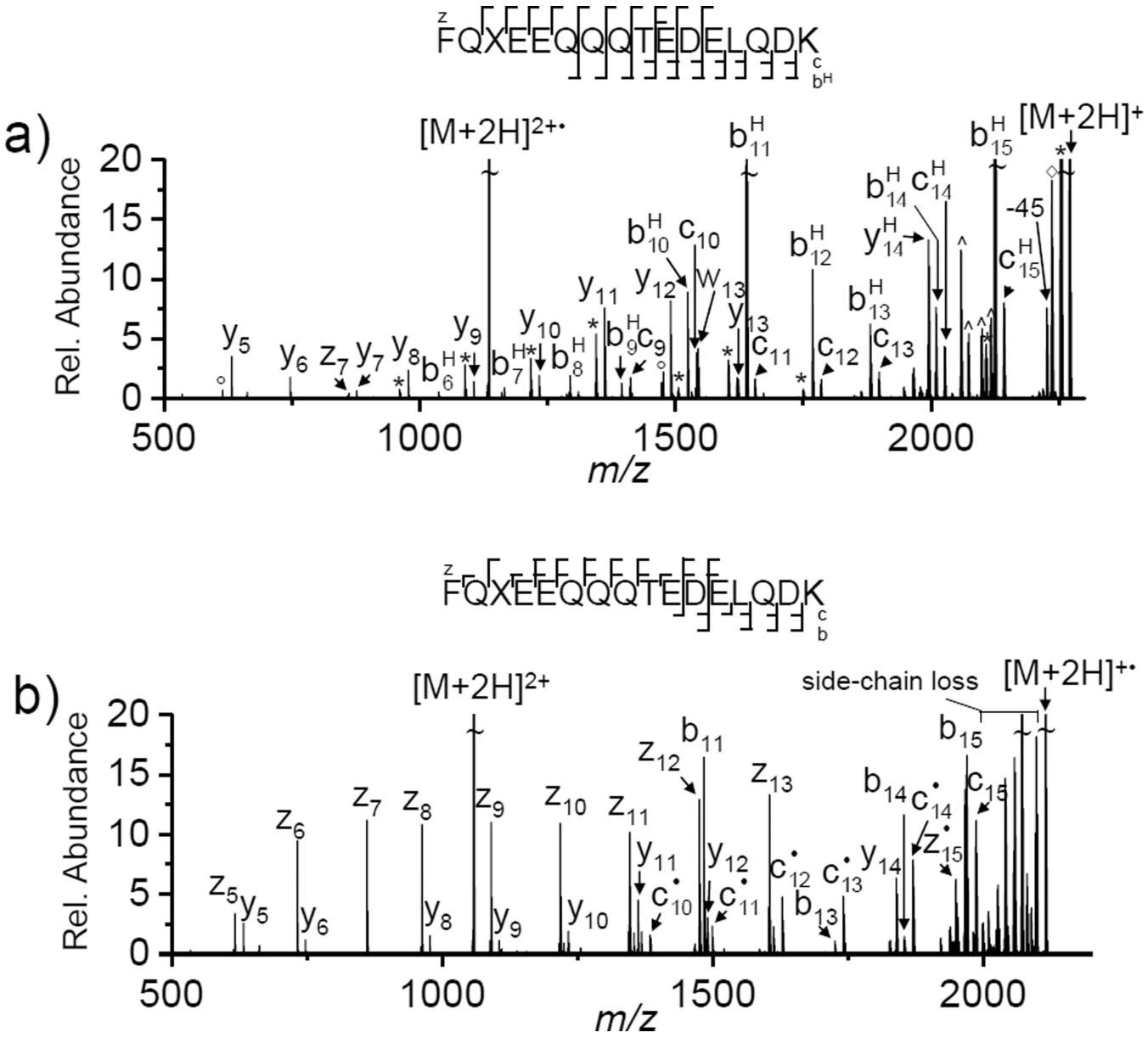


Figure 5.

ETcaD of TEMPO and acetamide tagged peptide dications ([**1** + 2H]$^{2+\bullet}$ and [**2** + 2H]$^{2+}$), FQXEEQQQTEDELQDK, where X is TEMPO (**1**) or acetamide derivatized amino acid (**2**). a) ETcaD of [**1** + 2H]$^{2+\bullet}$, and b) ETcaD of [**2** + 2H]$^{2+}$. *, °, ◊, and ^ indicate loss of NH_3, H_2O, (NH_3 + H_2O), and TEMPO related fragments, respectively. Superscript "H" on the upper right-hand side of b, c and y ions indicates +1 Da shift by abstraction of a labile hydrogen atom.

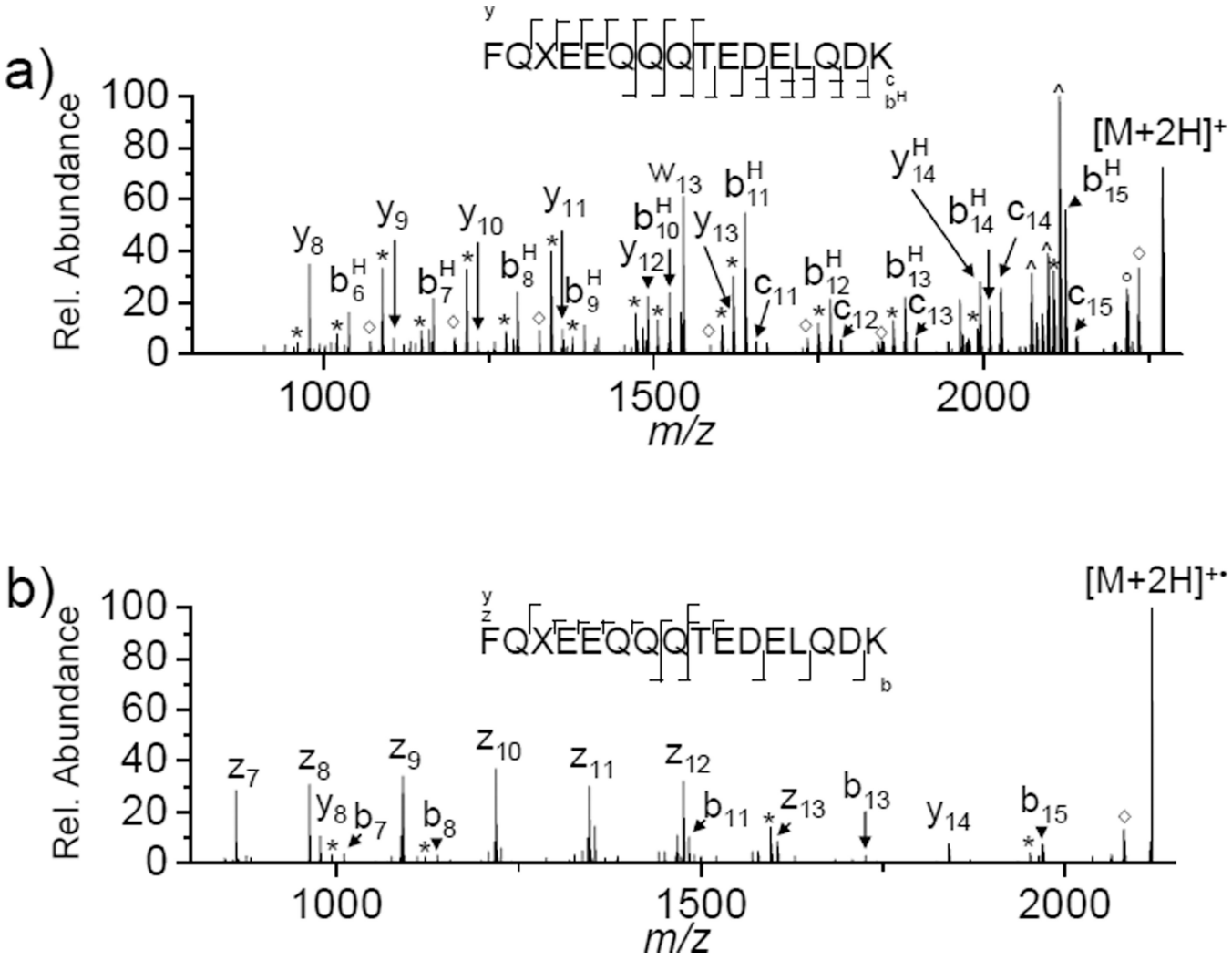


Figure 6.

CID of the charge-reduced TEMPO and acetamide tagged peptide cations ([**1** + 2H]$^{2+\bullet}$ and [**2** + 2H]$^{2+}$), FQXEEQQQTEDELQDK, where X is TEMPO (**1**) or acetamide derivatized amino acid (**2**). a) ETcaD of [**1** + 2H]$^{2+\bullet}$, and b) ETcaD of [**2** + 2H]$^{2+}$. *, °, ◊, and ^ indicate loss of NH_3, H_2O, (NH_3 + H_2O), and TEMPO related fragments, respectively. Superscript "H" on the upper right-hand side of b, c and y ions indicates +1 Da shift by abstraction of a labile hydrogen atom.

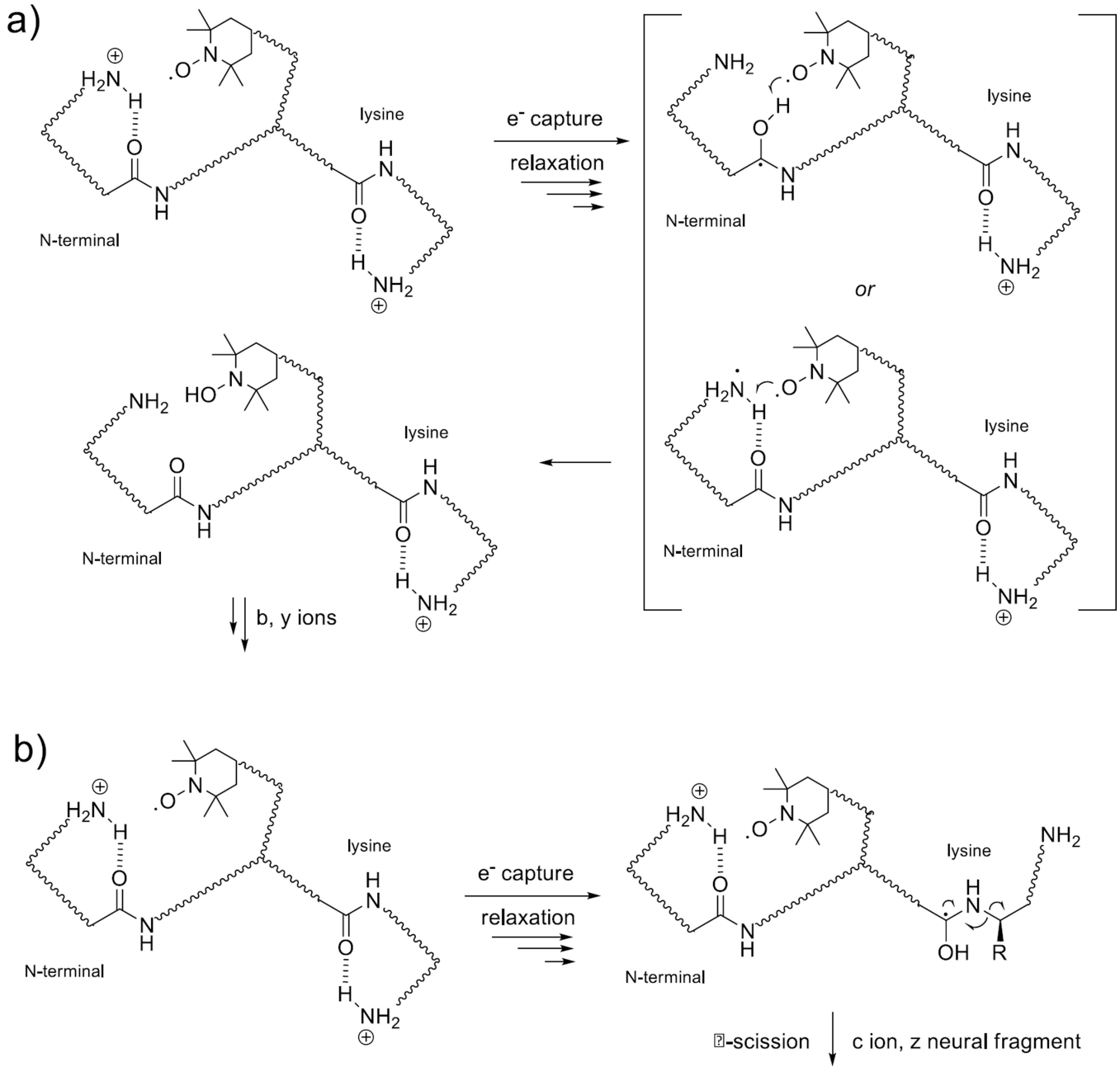
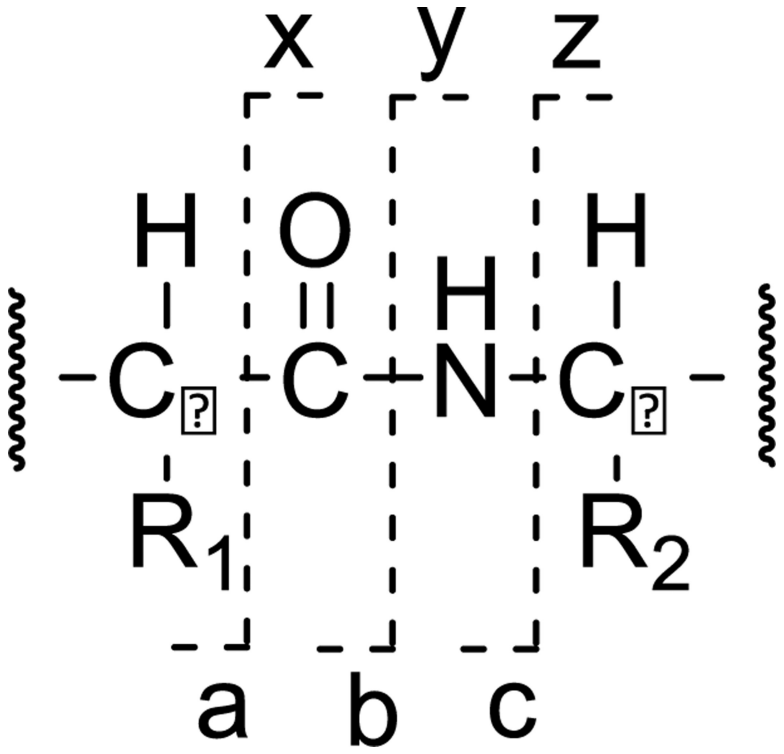


Figure 7.

The reaction mechanisms of ETcaD of the TEMPO labeled model peptide dication, [**1** + 2H]$^{2+\bullet}$. a) b and y type ions are formed by abstracting a hydrogen atom by the TEMPO nitric oxide radical from either a hypervalent amine or an aminoketyl radical, followed by amide backbone fragmentation. b) c ion and z neural fragment are formed by electron recombination in the protonated lysine side-chain, followed by β-scission via an aminoketyl intermediate.



Scheme 1.

Table 1

Electron Affinity, Proton Affinity, Hydrogen Atom Affinity of TEMPO

Method	Electron Affinity (eV)	Proton Affinity (kJ/mol)	Hydrogen Atom Affinity (kJ/mol)
B3LYP[a]	0.361	893.1	267.9
BMK[a]	0.296	890.7	278.6
M05-2X[a]	0.412	886.0	284.2
MP2[a]	0.644	874.9	296.1
PMP2[a]	0.529	873.0	285.0
B3-MP2[a,b]	0.503	884.0	282.0
B3-PMP2[a,b]	0.445	883.1	276.4
Experimental	N/A	882.3	N/A

[a]6-311++G(3df,2pd) basis set was used for electronic energy calculation. Initial geometry was optimized at the B3LYP/6-31+G(d,p) level and used for further electronic energy refinement. Enthalpy was calculated by thermochemical parameters obtained from vibrational frequencies at 1 atm and 298.15 K.

[b]Simple average of B3LYP and MP2 or Projected energy of MP2 (PMP2).