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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 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.

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).

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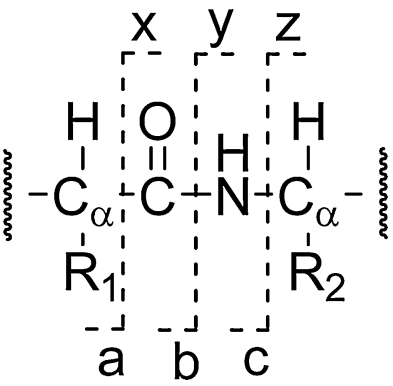

Scheme 1.

For more than a decade, experimental and theoretical studies have attempted to illuminate the mechanisms 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 [28–34] and modified peptide backbones [35–39]. 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 [40]. However, even without a mobile electron or hydrogen atom, ECD backbone fragmentation processes are still observed [41], 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 (Fig. 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.

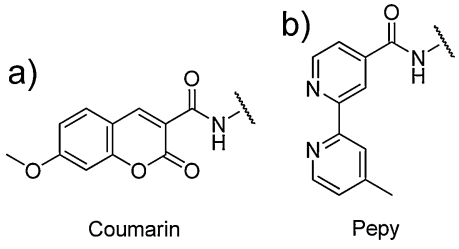

Fig. 1. Structures of (a) coumarin and (b) pepy groups used for the spin traps by O'Connor and Turecek groups [28,29]. Both functional groups are conjugated to the N-terminal or the lysine ε-amine of the model peptides via amide coupling chemistry employing N-hydroxysuccinimine (NHS) activated reagents.

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 (Fig. 2). Of particular interest is that the TEMPO group has the fairly similar proton affinity (882 kJ/mol) [42] 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 (FQpSEEQQQT-EDELQDK) 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 into dehydroalanine (dA) under basic solution conditions by adding 20 μL of 0.3 N $Ba(OH)_2$ and incubating for 1 h at room temperature. A 1 μL portion of 1,2-ethanedithiol was subsequently added to the reaction