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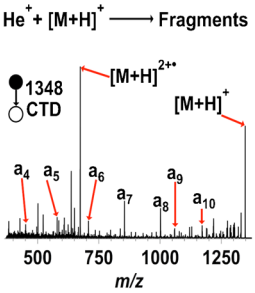
RESEARCH ARTICLE

Charge Transfer Dissociation (CTD) Mass Spectrometry of Peptide Cations Using Kiloelectronvolt Helium Cations

William D. Hoffmann,[1] Glen P. Jackson[1,2]

[1]Department of Forensic and Investigative Science, West Virginia University, Morgantown, WV 26506, USA
[2]C. Eugene Bennett Department of Chemistry, West Virginia University, Morgantown, WV 26506, USA



Abstract. A kiloelectronvolt beam of helium ions is used to ionize and fragment precursor peptide ions starting in the 1+ charge state. The electron affinity of helium cations (24.6 eV) exceeds the ionization potential of protonated peptides and can therefore be used to abstract an electron from—or charge exchange with—the isolated precursor ions. Kiloelectronvolt energies are used, (1) to overcome the Coulombic repulsion barrier between the cationic reactants, (2) to overcome ion-defocussing effects in the ion trap, and (3) to provide additional activation energy. Charge transfer dissociation (CTD) of the $[M+H]^+$ precursor of Substance P gives product ions such as $[M+H]^{2+\bullet}$ and a dominant series of a ions in both the 1+ and 2+ charge states. These observations, along with the less-abundant a + 1 ions, are consistent with ultraviolet photodissociation (UVPD) results of others and indicate that C–C^{α} cleavages are possible through charge exchange with helium ions. Although the efficiencies and timescale of CTD are not yet suitable for on-line chromatography, this new approach to ion activation provides an additional potential tool for the interrogation of gas phase ions.

Keywords: Dissociation methods, Charge transfer dissociation, Peptide fragmentation, Ion chemistry

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Introduction

Tandem mass spectrometry (MS/MS) has been a core technology in the development of proteomics, metabolomics, and other branches of biomedical research [1]. MS/MS is most commonly accomplished through collision-induced dissociation (CID), which relies on the conversion of kinetic to internal energy through ion/molecule collisions [2, 3]. Oftentimes, CID does not provide complete fragmentation of the peptide backbone and results in significant side-chain losses, including the loss of post-translational modifications, and thereby complicates the interpretation of tandem mass spectra [4, 5]. These limitations have fueled a significant investment in alternative fragmentation techniques, including electron transfer dissociation (ETD) with cationic [6–9] or anionic [6, 10] precursor ions, electron capture dissociation (ECD) with cationic [9, 11], or anionic precursor ions [12], photodissociation [13–20], metastable atom-activated dissociation (MAD) [21–28], electron ionization dissociation (EID) [29], and electron detachment dissociation (EDD) [30]. Each technique has its merits and limitations. Photodissociation techniques require a chromophore that can absorb at the incident wavelength to initiate fragmentation, and such chromophores can be relatively nonselective amide bonds [13–20] or highly site-selective [31–33]. Chromophores can also include specific and native chromophores like disulfide bonds [34] but non-native chromophores are dependent on the ability to chemically modify the peptides or proteins of interest.

Although ETD/ECD fragmentation occurs on a timescale fast enough to prevent hydrogen scrambling, these techniques are typically limited to the fragmentation of multiply charged precursor ions ($z \geq 2+$). For example, McLuckey and coworkers have shown that non-dissociative electron/ion recombination becomes the dominant process as charge state decreases [35–37]. Because ETD/ECD requires multiply charged precursor ions, the 1+ and 2+ charge states will have the least efficient fragmentation [37]. Although activated ion ETD (aiETD) [38] and electron transfer collisional activated dissociation (ETcaD) [39] provide better sequencing results for 2+ precursor ions, there remains a relative dearth in fragmentation methods available to dissociate 1+ and 2+ ions, which tend to dominate tryptic digests [40–43].

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Correspondence to: Glen Jackson; *e-mail:* glen.jackson@mail.wvu.edu

To date, the majority of ion/ion dissociation techniques have relied on cation/anion interactions because of their favorable cross-sections, as described by the Landau-Zener equation [8, 44]. Cation/cation reactions lie behind a Coulombic repulsion barrier of a few eV and are therefore difficult to achieve in quadrupole and linear ion traps [45]. However, Zubarev and coworkers recently described the use of a microwave air plasma to produce a variety of charged and neutral species for the dissociation of multiply charged angiotensin I and ubiquitin precursor ions [45]. The beam emerging from the microwave plasma chamber was accelerated to 1–2 keV to overcome the Coulombic barrier between the cationic reagents. The results showed a combination of charge reduction, charge increase, and dissociation with ions characteristic to CID and ECD reactions. Unlike Zubarev's report, which used an unknown mixture of reagent air cations such as $O_2^{+\bullet}$ and $N_2^{+\bullet}$, we herein use a pure helium-based ion gun to generate a beam of cations with a well-defined electron affinity (EA) of 24.6 eV, which is the largest of any 1+ ion—and considerably larger than the EA of $O_2^{+\bullet}$and $N_2^{+\bullet}$—so can drive reactions that are intractable through the use of reagents with smaller electron affinities.

Given sufficient kinetic energy to overcome the Coulombic barrier, one would expect a reaction between a target protonated peptide and helium cation to be:

$$[M + H]^+ + He^+ \rightarrow [M + H]^{2+\cdot} + He \rightarrow \text{fragments} \quad (1)$$

where the abstraction of an electron by the helium ion creates a hole on the analyte precursor ion, which drives radical fragmentation.

Helium cations have an electron affinity (24.6 eV) that greatly exceeds the ionization energy of singly protonated Substance P cations, which is approximately 10.6 eV [46]. Therefore, given sufficient energy to overcome the Coulombic barrier, the electron affinity helium cations should have at least 13 eV of excess energy above the ionization potential of protonated or doubly protonated Substance P [46], which is sufficient to fragment even the strongest covalent bonds. When the target precursor in Reaction 1 is a neutral, the analogous reaction is called dissociative charge transfer and has been extensively studied for small organic neutrals [47]. Following this lead, and the IUPAC recommendations for mass spectrometric terms [48], we have therefore adopted the term charge transfer dissociation (CTD) to describe this class of reactions. CTD of peptide anions would be similar to negative nETD [6, 10], except that additional translational energy available for reaction in CTD.

Experimental

Figure 1 shows a schematic of the instrument modification for accomplishing CTD. A full description of the instrument modifications and method is given in the Supplemental Material. Briefly, a saddle field fast ion/fast atom source (VSW/Atomtech, Macclesfield, UK), with the ion gun cathode in place, was interfaced to the ETD chamber of an LTQ Velos Pro (Thermo Electron Corporation, San Jose, CA, USA) mass spectrometer using a home-built vacuum chamber cover. A variable leak valve was used to control the flow of helium through the saddle field source. A 6 kV square-wave from a high voltage amplifier produced helium ions in synchrony with the portion of the scan function normally reserved for CID, similar to our previous MAD-MS experiments [25].

Results and Discussion

To verify the presence of He^+ ions with an electron affinity of at least 24 eV, we used the He^+ beam to conduct CTD (dissociative charge transfer) of a well-characterized volatile organic, trichloromethane. The resulting fragmentation spectrum is shown in Supplementary Figure S3 and the results are discussed in the Supplemental Material.

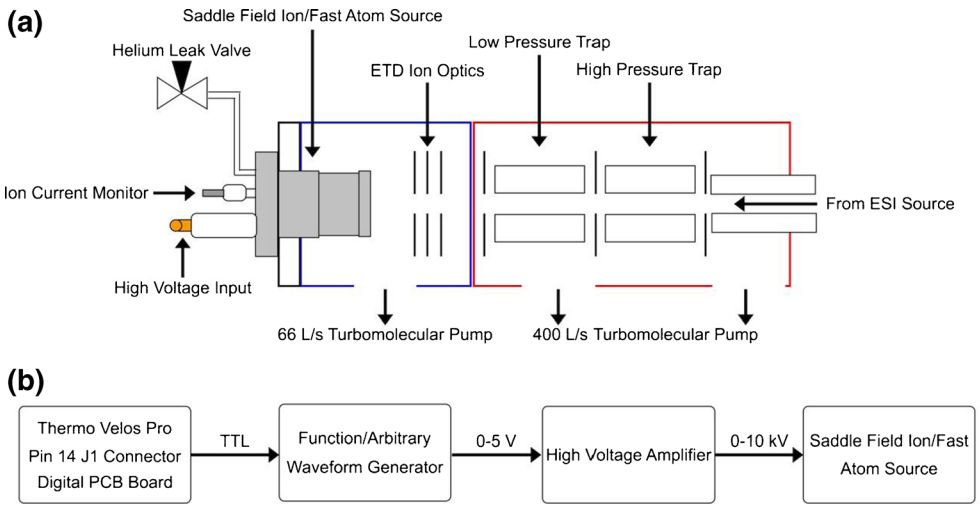

Figure 1. **(a)** Schematic representation of the experimental setup. **(b)** Box diagram of the signal flow and electronic components used to time and pulse the saddle field source

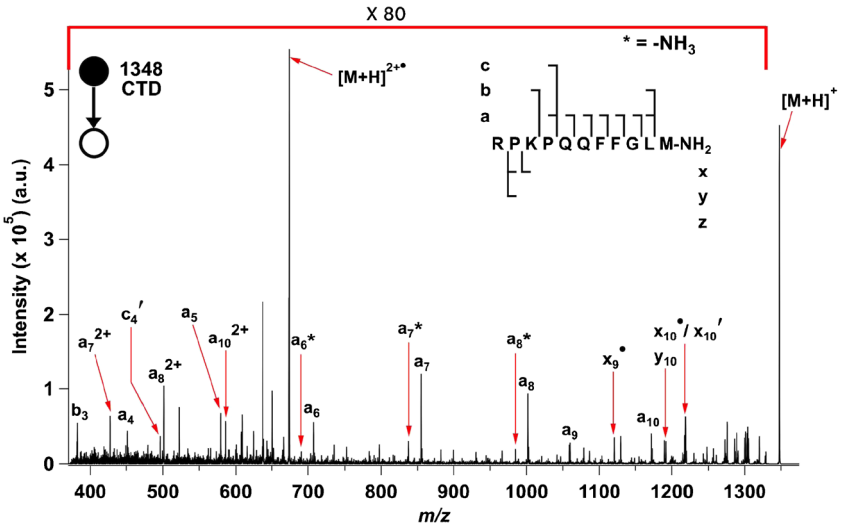

Figure 2. CTD Spectrum of 1+ Substance P. The 2+ radical is the major product with a dominant sequence of a-ions and less abundant b-, c-, x-, and y-ions. The region between *m/z* 370–1330 has been multiplied by 80 for clarity

Initial experiments used Substance P because it provides a well-characterized benchmark for the fragmentation of N–C_α bonds [49]. Solutions of 60 µM Substance P in 1:1 (v:v) MeOH:H_2O with 1% HOAc were directly infused through the ESI source and the singly protonated precursor ion was isolated with different isolation windows at *m/z* 1347.9 and subjected to CTD. At 1-s reaction times, fragmentation efficiencies were worse above and below 6 keV He^+ ions, and the CTD efficiency was below the S/N threshold with He^+ ions below 4 keV.

Figure 2 shows the CTD spectrum of 1+ Substance P averaged across 52 scans and activated with 6 keV He^+ ions for 980 ms. For clarity, the intensity from *m/z* 370–1330 has been multiplied by a factor of 80. A dominant peak at *m/z* 674.34 represents the expected charge transfer product shown in Reaction 1, $[M+H]^{2+\bullet}$. Fragments are dominated by a-ions, which result from the cleavage of the C–C_α bond with charge retention on the N terminus. The series of a-ions is also accompanied by a series of a-NH_3 ions. Reilly and coworkers saw similar fragmentation of singly protonated Substance P when using photodissociation and CID, but noted that the ammonia losses were most substantial when using post-source decay and low energy CID [50]. The predominance of a-type ions is not unusual when a peptide contains a basic residue at the N terminus [50] and the fragmentation pathways observed for CTD are similar to ultraviolet photodissociation (UVPD) and other high energy fragmentation pathways [51], including femtosecond laser induced

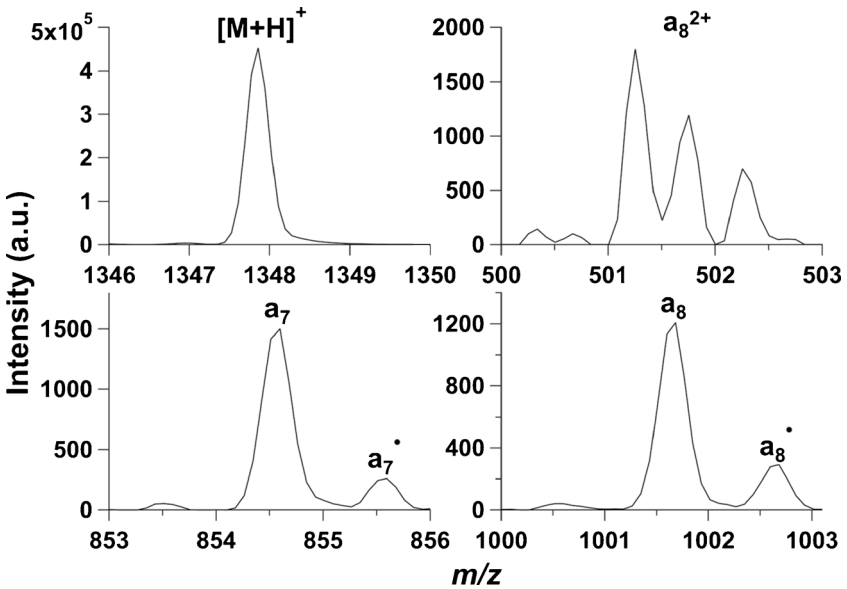

Figure 3. Isolated mass spectra showing the monoisotopic $[M+H]^+$ precursor along with the associated a_{7-} and a_{8-}ions. In both cases an $a_n + 1$ ($n = 7, 8$) ion is observed stemming from hemolytic cleavage of C – C_α. Subsequent loss of a hydrogen radical results in the even electron a-ions. The upper right panel shows the a_8^{2+}-ion observed using a precursor isolation window of *m/z* 4

dissociation [19] and EID [29]. Although the current experiment was performed at $q_z = 0.25$ as the precursor trapping parameter, future experiments need not be limited in q_z because the reagent ions are not stored or expected to be affected by rf amplitude in the ion trap. Because we are not storing or trapping the reagent helium cations, the q_z value could be reduced to effectively trap smaller mass/charge fragments.

In addition to the near-complete series of singly charged a ions, several doubly charged a ions were also produced. Widening the precursor isolation window and looking for the presence of the expected isotopic envelope assured the identifications of these doubly charged ions, although accurate mass measurements have not yet been performed to confirm these assignments. The full CTD mass spectrum resulting from opening the isolation window can be seen in Supplementary Figure S2.

Following CTD of the monoisotopic precursor, the singly charged a series ions are also accompanied by a+1 ions. The a + 1 ions are thought to arise from the homolytic cleavage of the C–C_α bond along the backbone [50]. Subsequent elimination of a hydrogen radical results in the formation of even electron a-type ions [15, 18]. Figure 3 shows the isolated monoisotopic precursor along with the a_7 and a_8 ions with the a + 1 ions marked. The same a + 1 ions have been seen with UVPD [18, 52], and metastable atom activated dissociation [25], when the peptide contains a basic residue at the N terminus. Conventional (low energy) CID of peptides containing a lysine residue at the N terminus shows poor yields of a ions [52], but UVPD was able to significantly improve the production.

Conclusion

The presence of these a + 1 ions in the CTD spectra indicates that there are radical mechanisms driving fragmentation. These types of radical driven mechanisms would be expected based on a single charge transfer from the helium cation to the peptide cation. Radical migration has been shown to be sensitive to the tertiary structure of proteins when charge states have significant impacts on the conformation [31]. For example, Julian and Diedrich show site-specific, radical driven cleavage at sites of phosphorylation when using UVPD at 266 nm [53], further underpinning the importance of radical driven reactions in the dissociation of 1+ peptide cations. CTD provides an alternative, high-energy method for the interrogation of low-charge-state gas phase ions.

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