

# Examining the Combined Features of ETD and CAD: Increased Peptide Identification

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## Overview

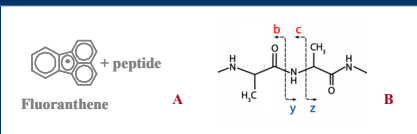
Here we examine the features of CAD and ETD in peptide fragmentation and identification in a proteomics platform using Mascot scoring and the MS/MS database.

## Introduction

Shotgun proteomics is the technique of choice for protein identification in complex samples. Confident peptide identification via database searching relies on quality MS/MS fragmentation. Peptides from enzymatic digestions vary dramatically in MW. High molecular weight or excessively basic peptides often exist in charge states  $> 2^+$  unsuited for high scoring CAD fragmentation.

Alternative fragmentation techniques exist, though their scope is currently less encompassing. The most promising of these alternative techniques is electron capture dissociation (ECD) and its counterpart, electron transfer dissociation (ETD). ETD results in the formation of c and z fragment ions. In this study we compare and contrast the utility of ETD and CAD in peptide identification via Mascot database searching.

**Scheme 1.** A. Fluoranthene anion used as the electron donor in ETD. B. The peptide backbone and the fragment ions created during vibrational (CAD, b, y ions) or electronic excitation (ETD, c, z ions).



Traditionally, CAD excitation has been used as the primary tool in peptide fragmentation. Peptide collision with inert gas ( $N_2$  or He) creates vibrational energy which is redistributed over the entire molecule. The weakest bonds commonly dissociate: in the peptide backbone this is the C-N bond (Scheme 1, b and y ions); among these bonds, those between select amino acids (e.g. N-terminal to proline) are particularly prone to dissociation.

In contrast to vibrational excitation is electronic excitation, which proceeds on a significantly faster time scale. In the case of ETD the anion fluoranthene donates an electron to the positively charged peptide cation. The mechanism that pursues is much debated, but the outcome is the dissociation of the N-C $\alpha$  bond creating N-terminal c ions and C-terminal z<sup>+</sup> ions.

## Methods

Protein samples were excised from a Coomassie-stained 1-D SDS PAGE gel. The samples were then reduced with DTT alkylated using acrylamide, and digested in gel with trypsin followed by peptide extraction. The peptides were then separated by a nano LC system and were directly eluted into a LTQ (linear ion trap) mass spectrometer equipped with ETD (Thermo Fisher). The data dependent acquisition of the mass spectrometer was set to perform MS/MS (both CAD and ETD) on the top 3 most intense ions from the survey scan.

MS instrument settings:

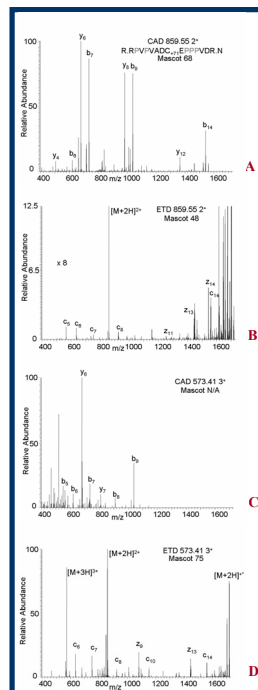
Full AGC Target: 30000.00  
SIM AGC Target: 10000.00  
MSn AGC Target: 10000.00  
CAD settings @ 35, max inject time 100 ms  
ETD settings: Anion AGC Target 200000, reaction time 50 ms, max cation ion injection 100 ms<sup>1</sup>.

Data were processed using Bioworks 3.3.1, which has the DTA extraction function distinguishing CAD from ETD scans (LTQ XL w/ ETD). CAD and ETD data were then separately searched against the MS/MS database using Mascot with identical settings, including 0.5 Da precursor and 1.2 Da fragment ion tolerance, except in the case of the ETD data where the instrument type parameter was set to ECD.

All peptides scoring less than the Mascot value where  $p \leq 0.05$  were discarded, followed by manual evaluation of all ETD spectra for goodness of fit, discarding all false positives.

## Results

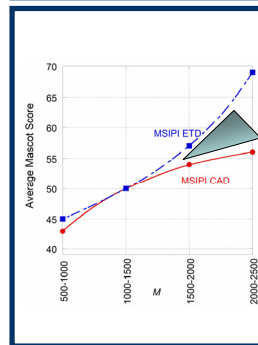
The stringency of manual validation of spectra intentionally left the final pool of peptides relatively small. This resulted in unambiguous confidence in all assignments. 105 peptides were identified by CAD and 67 peptides by ETD. Together there were 153 unique peptides, with only a small 12% overlap in peptide identifications and emphasizing the complementarities of the two fragmentation techniques<sup>2</sup>. These findings parallel what has been previously reported by Coon et al<sup>3</sup>. A demonstration of the typical work flow and experimental outcome can be seen in Figure 1 A-D. In Figure 3, the percentage of doubly and triply charged peptides as a function of the fragmentation technique is explored; some of the critical features in determining the 2<sup>+</sup>/3<sup>+</sup> ratio can be explained by the choice of enzyme.



**Figure 1.** MS/MS fragment ion spectra of the same proline rich peptide as dissociated by different techniques and at different charge states.

Note, even proline rich peptides where cleavage N-term to proline remains undetected in ETD still results in significant Mascot scores in both the 2<sup>+</sup> and 3<sup>+</sup> charge states.

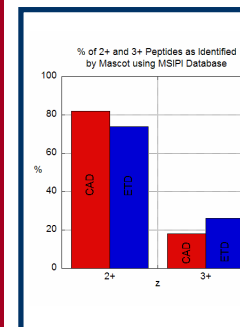
- CAD spectrum of the 2<sup>+</sup> peptide, where the major dissociation pathway is through proline
- ETD spectrum of the 2<sup>+</sup> peptide
- CAD spectrum of the 3<sup>+</sup> peptide, which does not result in a Mascot score above the threshold
- ETD spectrum of the 3<sup>+</sup> ion, resulting in the high Mascot score of 75



**Figure 2.** ETD and CAD results binned by molecular mass and Mascot score.

Average Mascot scores for peptides in four mass ranges<sup>4</sup>. The advantages of ETD at these anion concentrations and reaction times becomes apparent above  $M = 2000$  Da. At higher  $M$  ranges, peptides can be expected to carry more charge, which leads to increased fragmentation efficiency in ETD.

<sup>4</sup>Peptides  $> 2500$  Da were identified, but were too few for good statistics.



**Figure 3.** Peptides sorted by fragmentation type and charge state.

Although more than three times as many doubly charged than triply charged peptides were identified by ETD, in all cases when the same peptide was picked in different charge states, the higher charge state always produced a higher Mascot score.

This phenomenon is not attributable in CAD results. It should also be mentioned that the 2<sup>+</sup> to 3<sup>+</sup> ETD ratio was of particular surprise, in contrast to previously published results<sup>1</sup>; these observations are currently being resolved<sup>5</sup>.

## Conclusions

- CAD and ETD used in parallel increase the number of peptide hits in a shotgun proteomics platform.
- The overlap of homology amongst peptides positively identified by both fragmentation techniques was small (12%).
- Proline rich peptides, which often do not score high by Mascot with CAD excitation, can achieve above threshold scores with ETD.
- Above the molecular weight of 2000 Da, ETD is advantageous compared to CAD.
- With the current fluoranthene anion AGC values, along with precursor ion AGCs and reaction time, 2<sup>+</sup> peptides are preferentially fragmented by ETD.

## References

- Good, DM; Wirtala, M; McAlister, GC; Coon, JJ. 2008. Performance characteristics of electron transfer dissociation mass spectrometry. *MOLECULAR & CELLULAR PROTEOMICS* 6 (11): 1942-1951.
- Nielsen, ML; Savitski, MM; Zubarev, RA. 2005. Improving protein identification using complementary fragmentation techniques in Fourier transform mass spectrometry. *MOLECULAR & CELLULAR PROTEOMICS* 4 (6): 835-845.
- How To Maximize the ETD MS/MS Duty Cycle for Shotgun Proteomics. Russell, JD; Swaney, DL; Coon, JJ. 56<sup>th</sup> ASMS, 2008, Denver CO.

## Acknowledgements

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This poster may be downloaded from the Stanford University Mass Spectrometry website at <http://mass-spec.stanford.edu/Publications.html>

