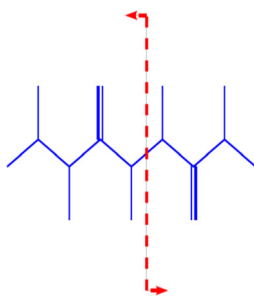APPLICATION NOTE

Peptide Sequence Analysis by Electron Transfer Dissociation Mass Spectrometry: A Web-Based Tutorial

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Abstract. We created a web-based tutorial designed to teach manual interpretation and identification of spectra acquired using electron transfer dissociation (ETD). The tutorial provides an explanation of the ETD fragmentation process with the goal of identifying all of the significant peaks in a spectrum. We discuss determination of the precursor mass and charge state, neutral losses, electron transfer without dissociation (ETnoD), and the mechanisms by which fragment ions are created. Our hope is to provide a tool that presents the information already taught in D.F.H.'s short courses in a way that is easy for any student or researcher in the mass spectrometry community to access. The tutorial may be found at http://www.huntlab.org.

Keywords: Electron transfer dissociation, ETD

Introduction

Electron transfer dissociation (ETD) is a relatively recent method for fragmentation of proteins using mass spectrometry [1, 2]. It is a particularly promising technique for the identification and characterization of phosphorylated peptides, highly charged peptides with multiple post-translational modifications and even intact proteins. In fact, inroads have been made in each of these areas. For example, ETD spectra have been used to characterize phosphorylated yeast proteins [3] and MHC class I peptides in cancerous and healthy cells [4, 5], to identify and characterize highly modified and large peptides from histones [6–8], and to obtain near complete coverage of intact proteins [9–13]. Udeshi et al. provide a protocol optimized for ETD data acquisition [10]. However, to realize the full potential of ETD, it is essential that the mass spectrometry community develop an in-depth understanding of ETD fragmentation patterns and how the resulting spectra differ from the more ubiquitous collisionally activated dissociation (CAD) fragmentation technique. We present a web-based tutorial designed as an educational tool that provides a detailed understanding of ETD fragmentation for the benefit of students and researchers. We also hope that it will be of help in improving the next generation of software tools for ETD analysis.

As is the case for CAD data, current instrumentation and acquisition methods allow for the collection of thousands or even tens of thousands of spectra in one run. Algorithms that aid in data analysis are essential. Analysis of ETD data presents many challenges. Most currently available algorithms were originally developed and optimized on CAD data, which differs from ETD data in significant ways. ETD produces different fragment types without the cleavage preferences found in CAD spectra. Also, post-translational modifications are stable when peptides are fragmented using ETD, resulting in fragments that shift by the masses of the modifications present. Furthermore, unlike CAD fragmentation, ETD is optimally suited for highly charged peptides created using digestion enzymes that are less typically used in CAD fragmentation. Most algorithms developed originally for use with CAD data were optimized using shorter tryptic peptides. All of this results in both false identifications and missed identifications when searching ETD data. Recent review articles highlight these issues [14–16], and one article reviewing de novo search algorithms for ETD data concludes that "expert knowledge will still be hampered by unexplained peaks within MS/MS spectra" for years to come [15]. In fact, many ETD search algorithms either ignore or eliminate peaks that might be useful for peptide identification, and published studies show that removing this rich information from ETD spectra actually enhances scoring from algorithms that cannot take it into account [17].

Efforts are already underway to improve search algorithm scoring for ETD data analysis. The ABRF iPRG 2011 study addressed this issue directly [18]. Several studies have addressed characteristics specific to ETD spectra, such as charge

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state dependencies resulting from the use of different enzymes [16] and neutral losses of amino acid side chains [19].

We believe that a better understanding of ETD fragmentation would benefit the mass spectrometry community by further educating researchers about characteristics specific to ETD spectra. This information could be used by algorithm developers continuing to fine tune both traditional search algorithms and de novo algorithms for ETD data. We are certain that the "unexplained peaks" can be explained and that these peaks may be used to provide direct information about peptide identification. A full understanding of ETD fragmentation can only be gained by taking a detailed look at individual spectra, even to the point of learning how to identify each peak manually. Toward that end, we have created a web-based tutorial designed to teach manual interpretation of ETD spectra with the hope that the community will use this information to better understand the data and may incorporate this understanding into algorithms designed for sequencing peptides and proteins.

Methods

The peptide used as an example in the tutorial was synthesized on an AAPPTEC model APEX 396 peptide synthesizer using standard fluorenylmethoxycarbonyl chloride (FMOC) chemistry. The data were acquired on a Thermo LTQ modified with a custom back-end ETD reagent ion source [1] by direct infusion using an Advion NanoMate HD chip-based peptide infuser. Both CAD and ETD data were acquired though only ETD data are presented in the tutorial.

Results and Discussion

Peptide Sequence Analysis by ETD Mass Spectrometry is a web-based tutorial designed to teach manual interpretation and identification of ETD mass spectra. The web platform allows us to edit or add new information easily, allowing the tutorial to evolve and grow. The tutorial can be found at http://www.huntlab.org.

The tutorial is designed to walk a student through the process of manually sequencing raw ETD spectra. Its central goal is to tie the interpretation of the peaks in a spectrum to the chemistry of the fragmentation process. An understanding of the underlying chemical process is essential to grasping the richness and complexity of an ETD spectrum. We aim to show the user that every significant peak in a spectrum has a chemical reason associated with its existence and that other aspects of the spectrum, such as peak abundance and the shapes of isotopic envelopes, are also important clues to the identity of a peptide.

The tutorial centers on manual interpretation of one triply charged peptide sequence acquired using ETD: SYKR AFAFSK. An averaged spectrum of this infused synthetic
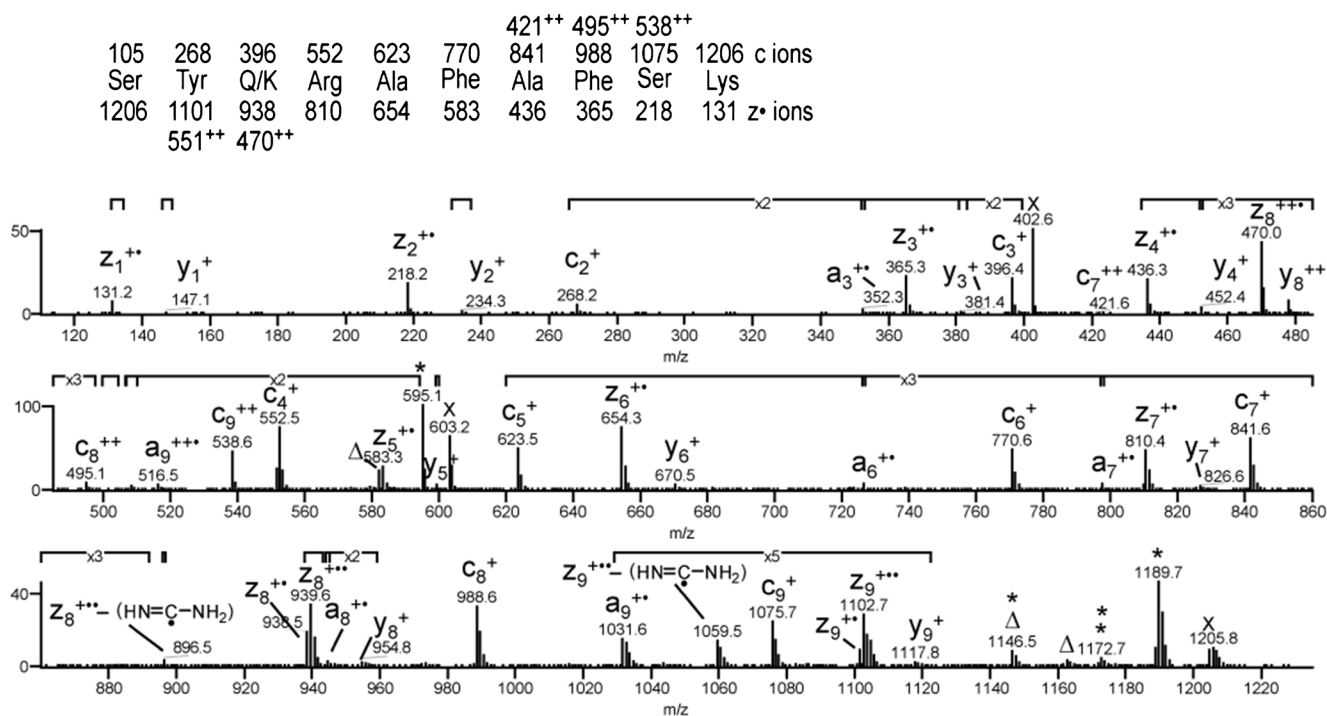

Fig. 1. Fully annotated sequence used as a teaching tool in the Electron Transfer Dissociation web tutorial. Included are c- and z·-ions as well as associated a·- and y-ions where present. Also shown are ETnoD products and neutral losses. **X** symbols mark residual signals at the m/z corresponding to the $(M + 3H)^{+++}$ ion, doubly charged ions that result from capture of one electron without dissociation, $(M + 3H)^{++·}$, and singly charged ions that result from capture of two electrons without dissociation, $(M + 3H)^{+··}$. * Denotes losses of NH_3 and **Δ** denotes the loss of $[NH = C · — NH_2]$

peptide is used to build the sequence manually by step by step description. This spectrum was chosen as a teaching tool to highlight the characteristics of ETD. Users are taken through an explanation of the mechanism of ETD fragmentation and the types of fragments that are created. The tutorial then focuses on identification of peaks in the spectrum that are unrelated to peptide fragmentation, including those attributed to the residual precursor, ETnoD, and neutral losses. Finally, we describe a method for determining the sequence of the peptide, identifying both singly and doubly charged c- and z·-type fragment ions and their associated a·- and y-ions. We show the user how to identify every significant peak in the spectrum to obtain a confident identification of the peptide. Figure 1 shows the fully annotated final product. Issues that complicate the analysis of ETD data are discussed, such as the lack of fragmentation at proline residues and distorted isotopic envelopes that occur as the result of doubly charged fragment ions that capture an electron but fail to dissociate.

Conclusion and Future Directions

It is our hope that users who complete our web tutorial will have a new understanding of ETD fragmentation and learn a method for confidently sequencing ETD spectra manually. In the future, we hope to add an interactive practice problem set to the tutorial to allow users to refine and apply the skills demonstrated. We will also add more complex examples to the tutorial since the interpretation of spectra becomes more difficult as precursor charge state increases and in the presence of post-translational modifications. Our ultimate goal is to provide examples of intact protein interpretation.

Acknowledgments

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