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Nota applicativa

Implementation of Efficient Electron Transfer Dissociation on a SYNAPT G2 HDMS System

Waters Corporation

Abstract

We demonstrate efficient electron transfer dissociation (ETD) fragmentation reactions on a SYNAPT™ G2 HDMS™ platform, using a simple and flexible source to introduce the ETD reagent nitrosobenzene. This solution provides routine high-resolution oa-ToF mass measurement of ETD generated fragment ions.

Benefits

SYNAPT G2 HDMS provides excellent sequence coverage and “top-down” ETD fragmentation of bovine ubiquitin for routine high-resolution oa-ToF mass measurement of fragment ions.

Introduction

Electron transfer dissociation is a radical-driven fragmentation technique which has found increasing use in the structural analysis of peptides and proteins. ETD results in cleavage of the peptide N-Cα bond, resulting in an even electron c fragment ion and an odd electron z• fragment ion. These fragments are complementary to those produced by collision induced dissociation (CID). Arguably the most significant advantage of ETD is retention of labile post-translational modifications, such as phosphorylation and glycosylation, on peptide backbone

fragments. In this technology brief we demonstrate, with specific examples, the implementation of ETD on a SYNAPT G2 HDMS platform.

Results and Discussion

The ETD reagent used to generate radical anions was nitrosobenzene (*m/z* 107). The reagent was held at room temperature. ETD experiments were performed on SYNAPT G2 HDMS instrument. In brief, the standard nanoFlow™ electrospray source was modified to incorporate a fast and efficient intermediate pressure glow discharge reagent anion source. During an ETD experiment, cations and anions are sequentially generated. Solutions of peptides were introduced into the nanoelectrospray ion source at a flow rate of 0.35 µL/min. The ion source polarity and the quadrupole set mass were sequentially switched to deliver triply- (or quadruply-) charged peptide cations and singly-charged nitrosobenzene radical anions (*m/z* 107) into the trap T-Wave™ ion guide. Analyte cations were generated in positive ion mode by applying approximately +3.4 kV to the nanospray emitter. Reagent anions were generated in negative ion mode by applying approximately -300 V to the glow discharge electrode relative to the sample cone voltage. The oa-ToF analyzer accumulated data at a rate of 1 ETD spectrum/sec. Reagent anion-trap T-Wave refill times were typically 100 msec, giving an analytical duty cycle of greater than 90%.

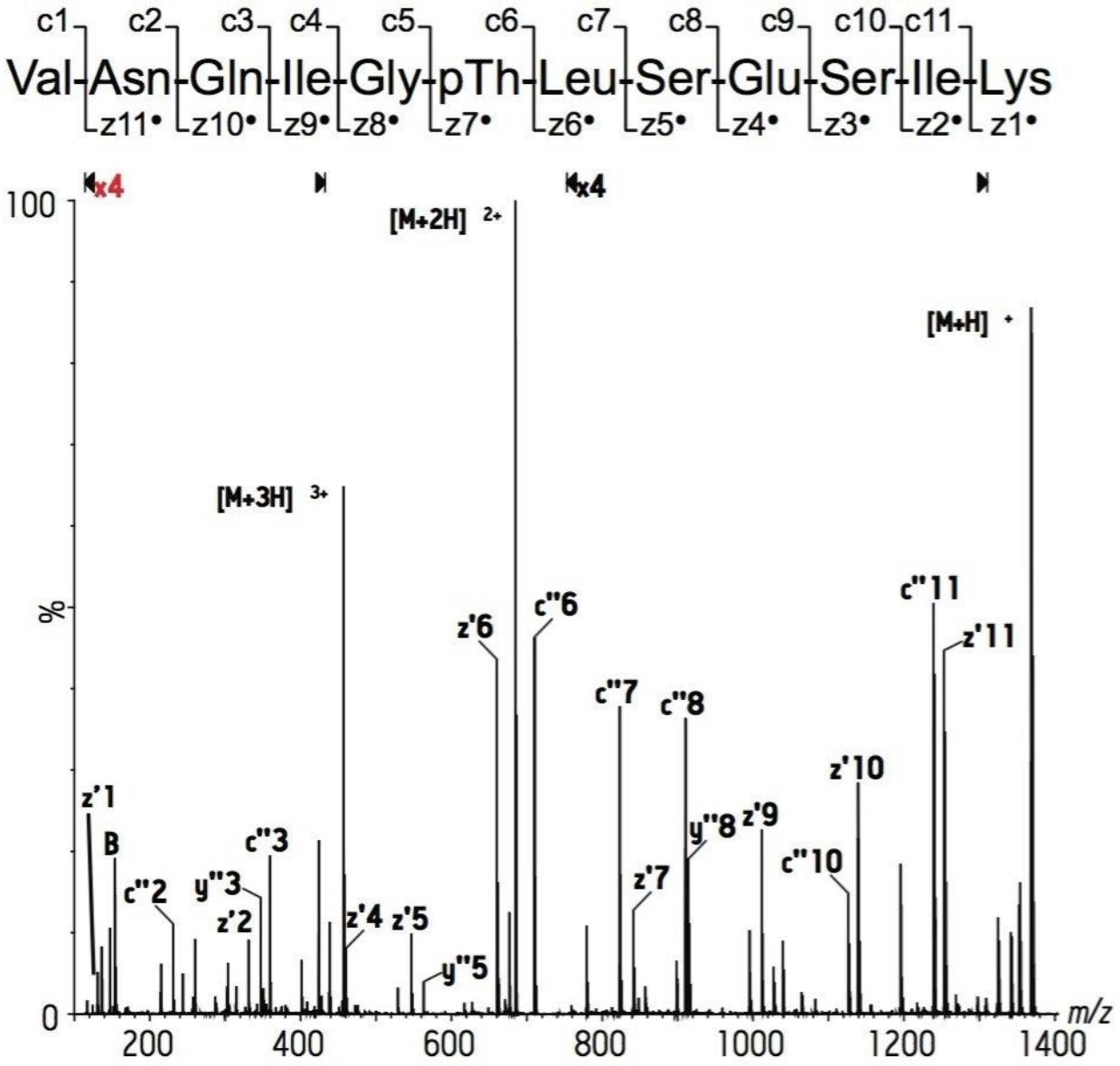

Figure 1. Electron transfer dissociation spectrum of the phosphopepitde VNQIG(pT)LSESIK. Precursor ion m/z 456.8^{3+} was selected for fragmentation.

Figure 1 shows the ETD fragmentation pattern of a phosphopeptide with excellent sequence coverage. The precursor ion was the $[M+3H]^{3+}$ ion, *m/z* 456.8. Under typical CID conditions the labile phosphate group would be lost as a neutral species, due to the slow heating process of CID. ETD is a far more rapid process, and as a result, the labile phosphate group remained attached to the amino acid residue, in this case threonine. This is an

important feature of ETD and greatly aids mapping of labile post-translational modifications.

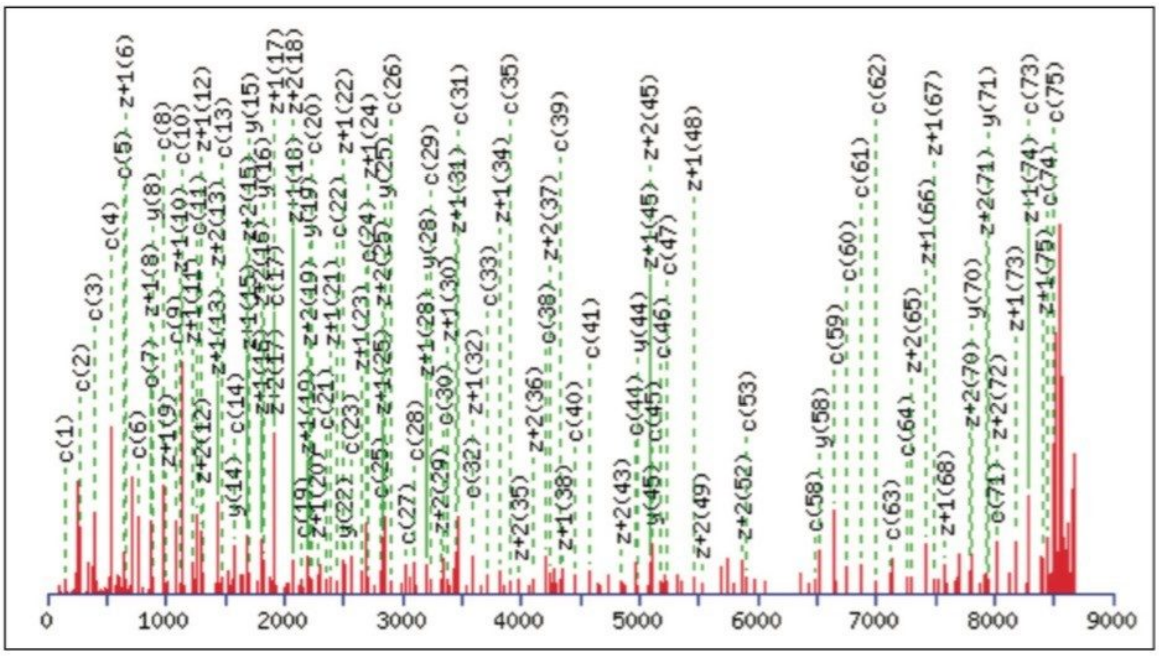

Figure 2. A MASCOT searched and annotated deconvoluted electron transfer dissociation spectrum of bovine ubiquitin. The precursor ion m/z 714.01^{2+} was selected for fragmentation.

Figure 2 shows a MASCOT searched and annotated deconvoluted spectrum of a "top-down" ETD fragmentation experiment of bovine ubiquitin. Deconvolution was performed using a probability-based algorithm.

Conclusion

Electron transfer dissociation on a SYNAPT G2 HDMS instrument has been demonstrated. We have shown excellent sequence coverage and efficient "top-down" ETD fragmentation of bovine ubiquitin (Mw 8561) and the retention of the labile phosphate moiety of a phosphopeptide.

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