

Small Neutral Losses in the Electron Capture Dissociation and Electron Transfer Dissociation of Nitrotyrosine-Containing Peptides

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Overview

We have previously shown [1] that electron capture dissociation (ECD) mass spectra of 3-nitrotyrosine-containing peptides are dominated by small neutral losses and proceeds via the electron predator mechanism [2].

Here we show that the losses are associated with the nitro modification and the N-terminus. Moreover, the results provide insight into the hierarchy of various proposed ECD mechanisms.

Introduction

Electron capture dissociation (ECD) has been hailed as a significant advance in the analysis of post-translational modifications; however we have previously shown that the presence of 3-nitrotyrosine within a peptide sequence has a deleterious effect on ECD backbone cleavage [1] which can be explained by the electron predator model proposed by Beauchamp and co-workers [2]. We also showed that the ECD mass spectra of nitrated peptides were dominated by the loss of small neutrals from the charge-reduced precursor.

Here, we have investigated the origins of those neutral losses in both ECD and electron transfer dissociation (ETD) by analysing both Lys- and Arg-containing peptides, peptides containing no basic amino acid residues (BAARs), N-terminal acetylated peptides and completing MS³ (IRMPD of ECD fragments), and show the potential of using these modifications for understanding the nature of several proposed ECD mechanisms.

Methods

GPLE_nYGFAK, GPLE_nYGFAR, GPLE_nYGFAL (where nY indicates 3-nitrotyrosine) and their unmodified counterparts were synthesised by Alta Bioscience, University of Birmingham. Selective N-terminus acetylation was completed by treating the peptides with acetic anhydride. High resolution ECD data were generated by use of a Thermo Finnigan LTQ FT Ultra mass spectrometer. ETD was performed on a Thermo LTQ Orbitrap Velos mass spectrometer. Data were manually analysed using Xcalibur 2.10 software. MS³ (IRMPD of ECD fragments) was performed on a Bruker 12 T Apex Qe Ultra, and analysed using DataAnalysis 4.0 software.

Conclusions

- As with Lys-containing peptides, ECD, ETD and saETD of Arg-containing nitrated peptides (Fig.1) proceeds via the electron predator model [2], i.e., little ECD backbone cleavage and extensive loss of neutral species is observed.
- ECD of nitrated peptides containing no basic amino acid residues (BAARs) (Fig.2) suggest that the NH₃ is lost from the BAAR, however the presence of b ions suggests that the Oslo mechanism may be taking place [3].
- ECD of N-terminal acetylated nitrated peptides (Fig. 3) contradicts the hypothesis that NH₃ is lost from the BAAR. However, as with non-BAAR-containing peptides b ions are being formed. These data indicate that if the positive charge is situated on the peptide backbone the mechanism for b-type ion formation [3] is favoured over the electron predator model.
- MS³ (IRMPD of ECD fragments) (Fig. 4) confirms the hypothesis that NH₃ loss is from the N-terminus and further suggests that the loss of •OH and H₂O following ECD of nitrotyrosine-containing peptides derive from the modified tyrosine.

References

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ECD, ETD and saETD mass spectra of nitrotyrosine-BAAR-containing peptides

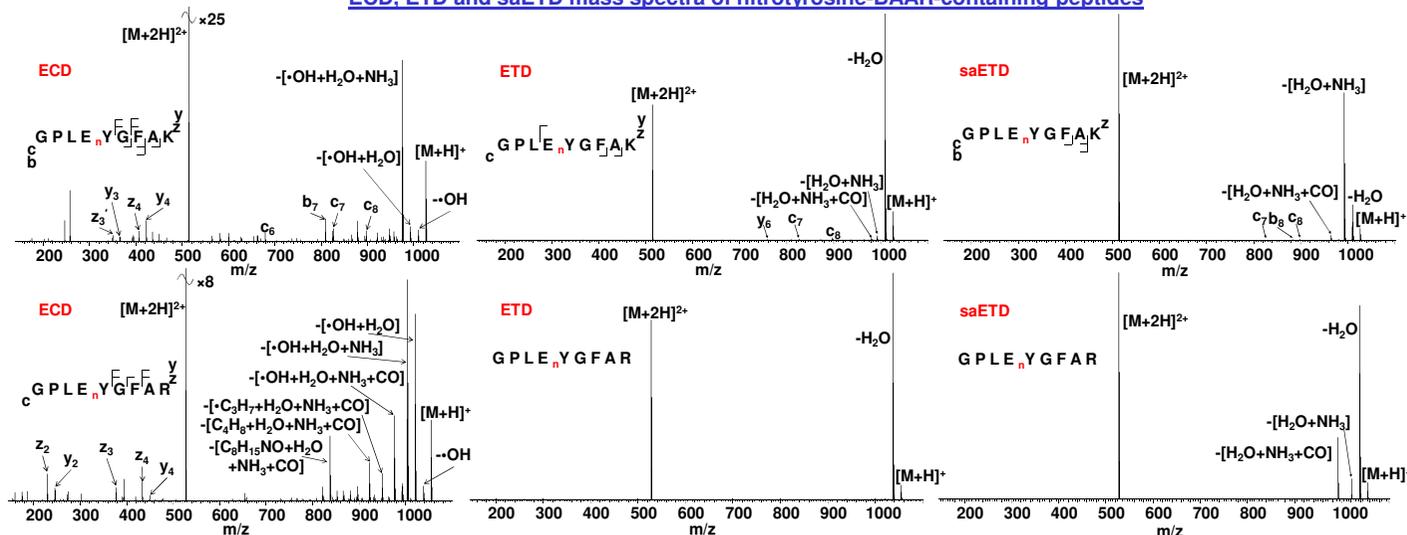


Fig.1: ECD, ETD and saETD of nitrated Lys/Arg containing doubly-charged peptides ions.

ECD mass spectra of modified and unmodified non-BAAR-containing peptides

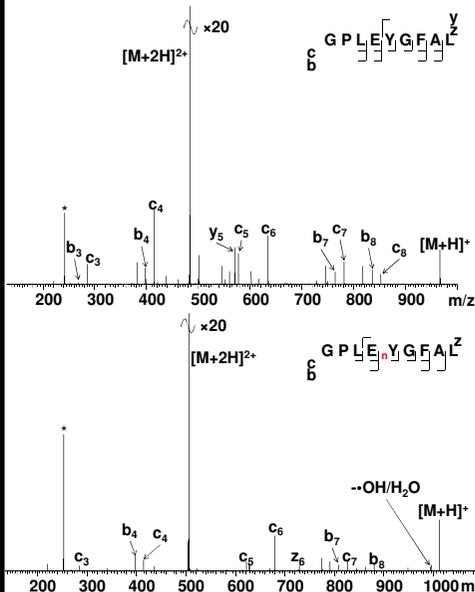


Fig.2: ECD of unmodified and nitrated non-BAAR-containing doubly-charged peptide ions.

ECD mass spectra of N-acetylated nitrated BAAR-containing peptides

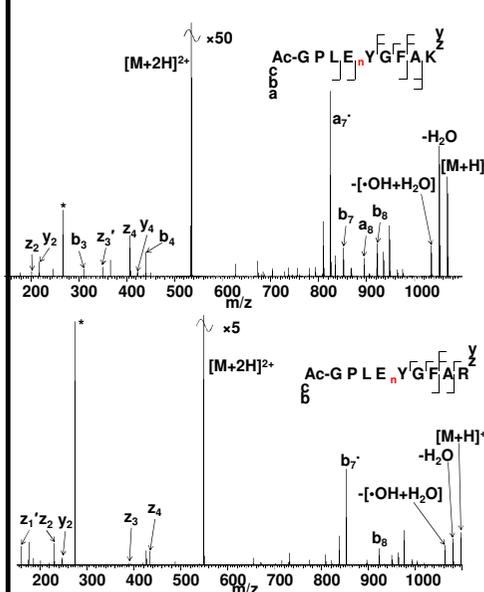


Fig.3: ECD of N-terminal acetylated nitrated BAAR-containing doubly-charged peptide ions.

MS³ mass spectra of nitrated BAAR-containing peptides

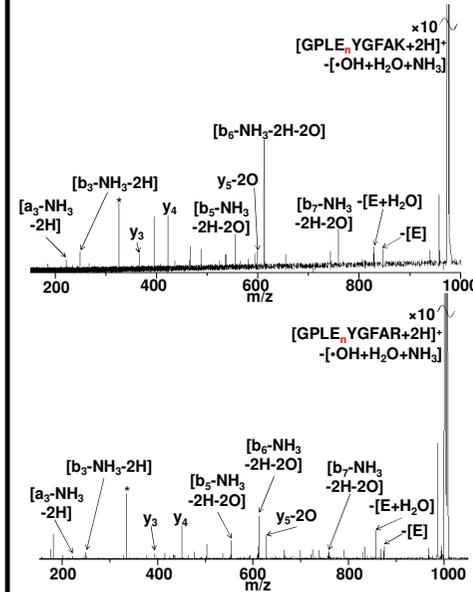


Fig.4: MS³ (IRMPD of ECD fragments) of nitrated BAAR-containing doubly-charged peptide ions.

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