Probing the Mechanisms of Electron Capture Dissociation Using Nitrotyrosine-Containing Peptides

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Overview

In vivo protein nitration occurs most commonly at tyrosine amino acid residues and is associated with many disease conditions that involve oxidative stress and inflammatory response.

In order to understand the mechanisms and consequences of protein nitration, it is necessary to identify nitrotyrosine-containing proteins and to localize the sites of modification.

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 show the potential of using these modifications for understanding the nature of the numerous proposed ECD mechanisms.

Methods

GPLEnYGFAK, GPLEnYGFAR, GPLEnYGFAL (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. Data were manually analysed using Xcalibur 2.10 software.

Conclusions

- As with Lys-containing peptides, ECD of Arg-containing nitrated peptides (Fig.1) proceeds via the electron predator model, i.e. little ECD backbone cleavage and extensive loss of neutral species.
- ECD of nitrated peptides containing no basic amino acid residues (BAARs) (Fig.2) proceeded as expected for non-modified non-BAAR-containing peptides [3], and no loss of small neutrals was identified.
- ECD of N-term acetylated nitrated peptides (Fig.3) also showed similar results to that of modified non-BAAR-containing peptides. Indicating 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.

Acknowledgements

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References

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2. Sohn, C. H., *et al.*, *Probing the mechanism of electron capture and electron transfer dissociation using tags with variable electron affinity,* JACS, 2009. **131**, 5444-5459
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ECD mass spectra of nitrated doubly charged peptides

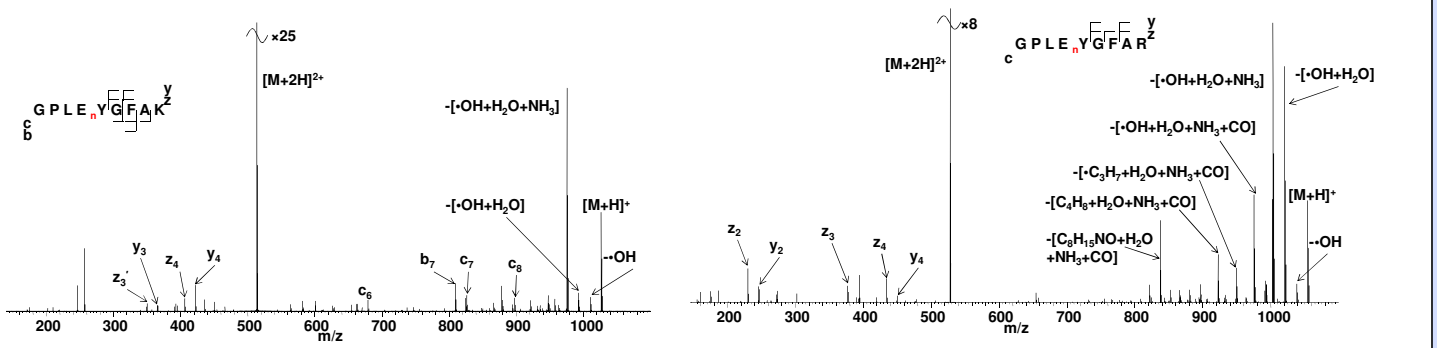

Fig.1: ECD of nitrated doubly charged peptide ions.

ECD mass spectra of non-BAAR-containing doubly charged peptides

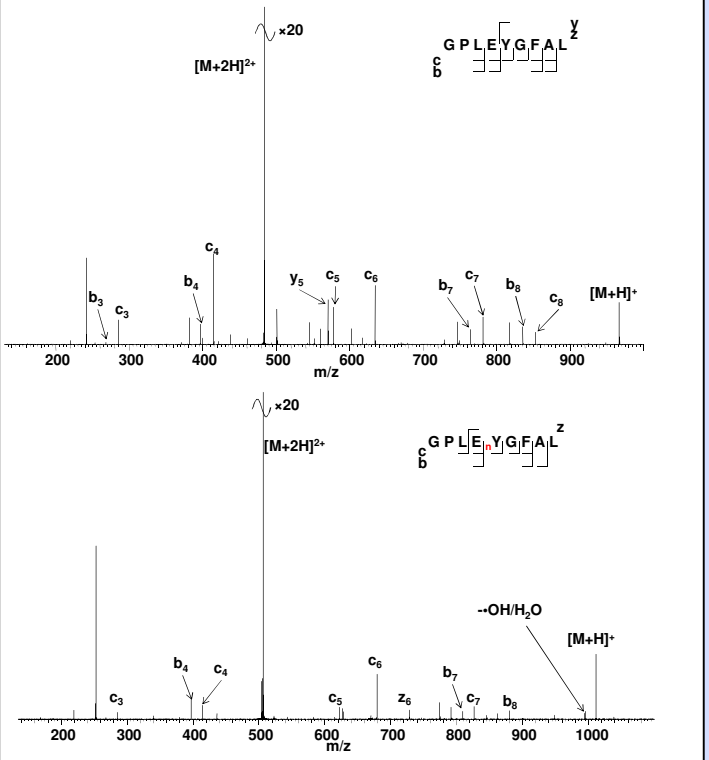

Fig.2: ECD of nitrated and non-modified non-BAAR-containing doubly charged peptide ions.

ECD mass spectra of N-acetylated nitrated doubly charged peptides

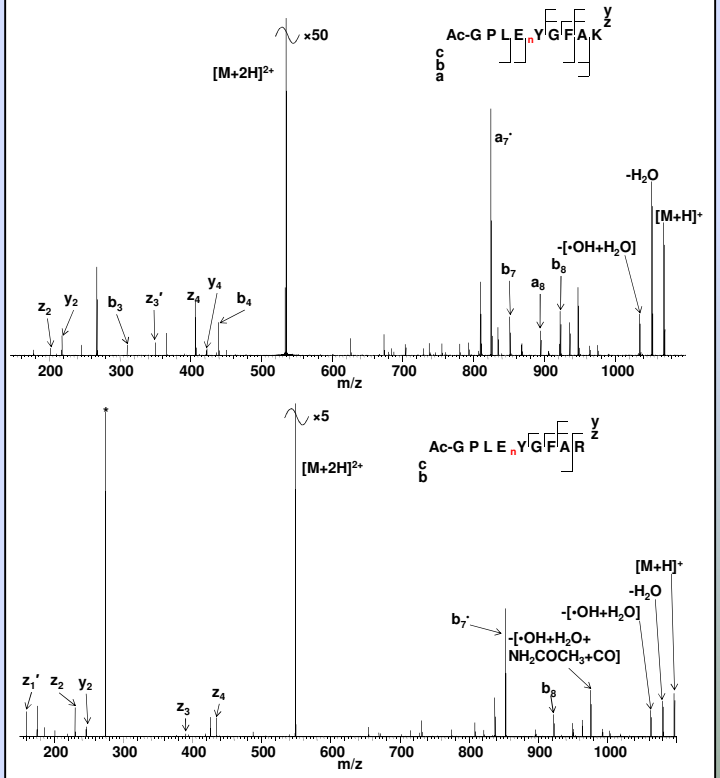

Fig.3: ECD of N-terminal acetylated nitrated doubly charged peptide ions.