The Presence of 3-Nitrotyrosine Affects the Site of Nitrosylation

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Overview

- *S*-nitrosylation (formed by the reaction of •NO and cysteine) is a ubiquitous post translational modification (PTM) and is fundamental for protein function and modulation[1]; whereas, tyrosine nitration (formed by the reaction of tyrosine and $ONOO^-$ (formed itself from $O_2^{-\bullet}$ and •NO)) is not fundamental for protein function and has been shown to modify specific protein function[2].
- Here we ask: Does the presence of 3-nitrotyrosine affect nitrosylation of cysteine in peptides when placed in oxidative conditions?

Introduction

- Both nitration (Tyr and Trp residues) and nitrosylation (Cys and Trp residues) are formed *in vivo* by the presence of •NO (with 3-nitrotyrosine also requiring a reaction with $O_2^{-\bullet}$ to form $ONOO^-$), and thus it is hypothesised that in higher oxidative conditions (presence of $O_2^{-\bullet}$) nitrosylation will be affected.
- We completed an *in vitro* study on four synthetic peptides based on a tryptic peptide of fibrinogen (NYCGLPGEYWLGNDK) which has been shown *in vivo* to undergo nitration at Tyr9.
- We have previously shown that nitrosylation of the non-nitrated peptide counterparts (*i.e.* NYCGLPGEYWLGNDK/R) results in nitrosocysteine formation exclusively[3].

Method

- NYCGLPGEnYWLGNDK, NYCGLPGEnYWLGNDR, NnYCGLPGEYWLGNDK and NnYCGLPGEYWLGNDR (where nY is 3-nitrotyrosine) were synthesised by Alta Bioscience (Birmingham, UK) and peptides&elephants (Potsdam, Germany).
- Peptides were incubated in 20 mM TRIS hydrochloride, 1 mM EDTA, 0.1 mM neocuproine and 1 mM GSNO at 37 °C for 30 min.
- Nitrosylated peptides were analysed with no further purification, and diluted to 2 pmol/µl in 49.5:49.5 % methanol:water, and 1 % formic acid.
- Samples were infused by use of an Advion Triversa Nanomate nanospray ionization source into a Thermo Fisher Scientific LTQ-Orbitrap Velos hybrid mass spectrometer, where CID data were generated.
- Data were manually analysed with Xcalibur 2.1 (Thermo Fisher Scientific) and searched for all theoretical fragment ions using ProteinProspector software (UCSF, USA).

Conclusions

- We show that when in the presence of 3-nitrotyrosine, in all cases, nitrosylation occurs at tryptophan residues.
- Formation of nitrosocysteine is still identified, *e.g.* y_{10} in Fig.1c.
- The nitrosocysteine:nitrosotryptophan ratio cannot be calculated, nor can a calculated estimate be proposed as the dominant peaks in all cases correspond to the neutral loss of •NO, which may be the result of either species.
- It is postulated from these data that *in vivo* in high oxidative conditions, such that 3-nitrotyrosine is present, typical *S*-nitrosylation functions may be affected *via* the competing formation of *N*-nitrosylation.

References

1. Hess, *et al*. Protein *S*-nitrosylation. Nat. Rev. Mol. Cell Biol. (2005). 6, 150-66

2. Abello, *et al*. Protein tyrosine nitration. J. Proteome Res. (2009). 8, 3222-38

3. Jones, *et al.* The radical ion chemistry of *S*-nitrosylated peptides. JASMS (2012). 23, 2063-74

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Results

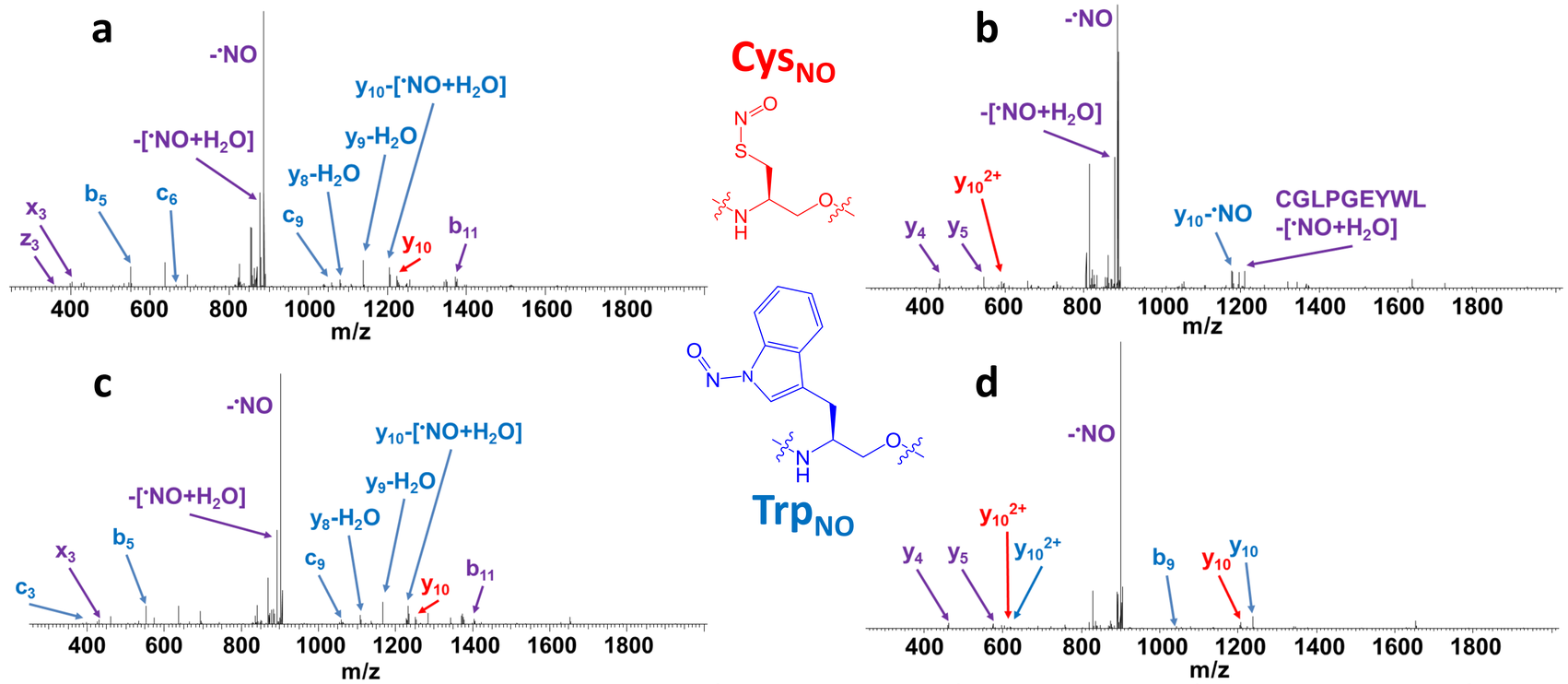

Figure 1: The CID mass spectra of the doubly-charged peptide ions a) $[NYCGLPGEnYWLGNDK + 2H]^{2+}$, b) $[NnYCGLPGEYWLGNDK + 2H]^{2+}$, c) $[NYCGLPGEnYWLGNDR + 2H]^{2+}$, and d) $[NnYCGLPGEYWLGNDR + 2H]^{2+}$ (nY indicates 3-nitrotyrosine). Peaks labelled in red correspond to ions containing nitrosocysteine, blue corresponds to nitrosotryptophan and purple do not distinguish between the two species.

Conclusions

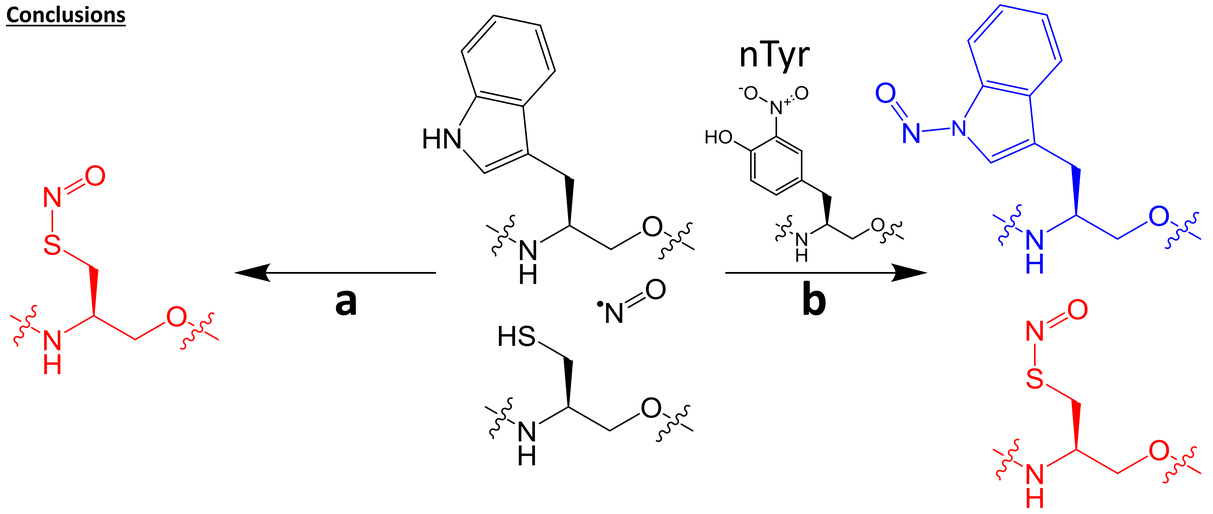

Figure 2:

a) In the presence of nitric oxide, cysteine residues will react resulting in the formation of nitrosocysteine exclusively. Tryptophan residues do not react with nitric oxide in a detectable manner.
b) In the presence of 3-nitrotyrosine (formed in high oxidising environments), nitric oxide reacts with both cysteine and tryptophan residues resulting in the formation of both nitrosocysteine and nitrosotryptophan, respectively.

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