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 modulation2; whereas, tyrosine nitration (formed by the reaction of tyrosine and NO3) is not fundamental for protein function and has been shown to modify specific protein function1.
- 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 O2- to form NO3); and thus it is hypothesised that in higher oxidative conditions (presence of O2-1) nitrosylation will be affected.
- We completed an in vitro study on four synthetic peptides based on a tyrosine peptide of fibrinogen (NYCGLPGEnYWLGNDK) which has been shown in vivo to undergo nitration at Tyr.
- We have previously shown that nitrosylation of the non-nitrosated peptide counterparts (i.e., NYCGLPGEnYWLGNDK/R) results in nitrosocysteine formation exclusively3.

Method

- NYCGLPGEnYWLGNDK, NYCGLPGEnYWLGNDK, NHYCGLPGeYfWLGNDK and NHYCGLPGeYfWLGNDK (where Nf is 3-nitrotyrosine) were synthesised at Alta Bioscience (Birmingham, UK) and peptides/animals (Potsdam, Germany).
- Peptides were incubated in 20 mM TRIS hydrochloride, 1 mM EDTA, 0.1 mM nucropine and 1 mM MIO3 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 ionisation 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).

Results

- We show that in the presence of 3-nitrotyrosine, in all cases, nitrosylation occurs at tryptophan residues.
- Formation of nitrosocysteine is still identified, e.g., y2 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.

Conclusions

References


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Figure 1: The CID mass spectra of the doubly-charged peptide ions a) [NYCGLPGEnfYWLGNDK + 2H]+, b) [NHYCGLPGeYfWLGNDK + 2H]+, c) [NYCGLPGEnYWLGNDK + 2H]+, and d) [NHYCGLPGeYfWLGNDK + 2H]+ (Nf 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.

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.