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Comparison of HNO reactivity with tryptophan and cysteine in small peptides

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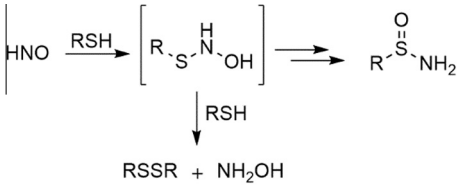
ABSTRACT

Recent discoveries of important pharmacological properties have drawn attention to the reactivity of HNO (azanone, nitroxyl) with biologically relevant substrates. Apart from its role in thiol oxidation, HNO has been reported to have nitrosative properties, for example, with tryptophan resulting in *N*-nitrosotryptophan formation. We have investigated the reactivity of HNO with tryptophan and small peptides containing either tryptophan or both a tryptophan and a cysteine residue. Our results point to the more reactive nature of cysteine towards HNO compared with tryptophan.

HNO (azanone, nitroxyl) is the protonated, one-electron reduced form of NO (nitric oxide), with its own important biological and pharmacological properties.[1–3] It has received significant attention due to its positive inotropic/lusitropic effects in normal and failing hearts, making it a potential heart failure therapeutic.[4,5] Moreover, we and others have shown that HNO targets several proteins involved in Ca^{2+} cycling.[6–9] Recent reviews also highlight the possible benefits of HNO in conditions such as vascular dysfunction, cancer, and alcoholism.[2,3] Thiols constitute one of the major biological targets of HNO.[3,10,11] Although HNO and NO are redox siblings, the thiol reactivity of HNO differs significantly from that of NO and normally results in the formation of a disulfide or a sulfinamide depending on the concentration of thiol (Scheme 1).[10,11] Additionally, recent reports point to the nitrosative role of HNO in generating *N*-nitrosoindole species (Scheme 2).[12,13] Although HNO-derived *N*-nitrosotryptophan (TrpNO) formation might be important in HNO pharmacology, information about this reactivity is scarce.

To gain more insight into HNO reactivity, we have investigated TrpNO formation in the presence of a nearby cysteine residue. For this purpose, we used the synthetic peptide, AGSCWA, which includes the active site sequence of the cysteine protease, papain, as well as several of its variants (CGSAWA, ACSAWA, AGCAWA, AGSAWC, GSAWCA, and AGSAWA). The small molecule *N*-acetyl-L-tryptophan was also employed for comparison.

As seen in Figure 1, treatment of the non-cysteine-containing peptide, AGSAWA, with different HNO-donors (Angeli's salt (AS) or *N*-hydroxy-2-(methylsulfonyl)benzenesulfonamide (2-MSPA)) at physiological pH and temperature results in the formation of the corresponding TrpNO-containing peptides (*m*/*z* 591) (Fig. 1a–c). Due to the reported instability of TrpNO species to mass spectrometry conditions,[14] electrospray ionization mass spectrometry (ESI-MS) data could be obtained only at relatively low temperatures (110 °C). Upon treatment of the sample with the HNO-donor byproducts (nitrite or 2-(methylsulfonyl)benzenesulfinic acid (2-MSSA)) no TrpNO species are detected. Also, the use of a ^{15}N-labeled HNO-donor, ^{15}N-2-MSPA, provides the corresponding Trp^{15}NO-containing peptide (*m*/*z* 592) (Fig. 1d). Similarly, the characteristic TrpNO peak was observed by UV–visible spectroscopy at 335 nm upon incubation of *N*-acetyl-L-tryptophan with the HNO



Scheme 1. Reaction of HNO with thiols.

Abbreviations: ACN, acetonitrile; AS, Angeli's salt; 2-BrPA, 2-bromo-*N*-hydroxybenzenesulfonamide; DMSO, dimethyl sulfoxide; DTNB, 5,5′-dithiobis(2-nitrobenzoic acid); DTPA, diethylenetriamine pentaacetic acid; ESI-MS, electrospray ionization mass spectrometry; FT-NMR, Fourier-transform nuclear magnetic resonance; HPLC, high pressure liquid chromatography; 2-MSPA, *N*-hydroxy-2-(methylsulfonyl)benzenesulfonamide; 2-MSSA, 2-(methylsulfonyl)benzenesulfinic acid; TFA, trifluoroacetic acid; TrpNO, *N*-nitrosotryptophan.

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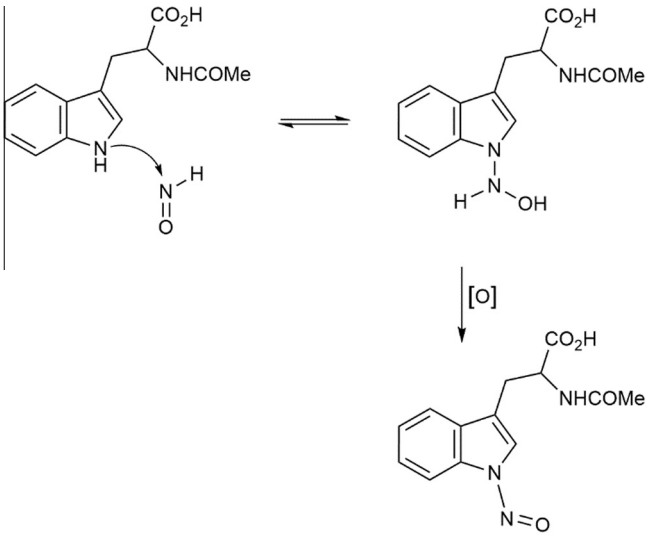

Scheme 2. Reaction of HNO with tryptophan.[13]

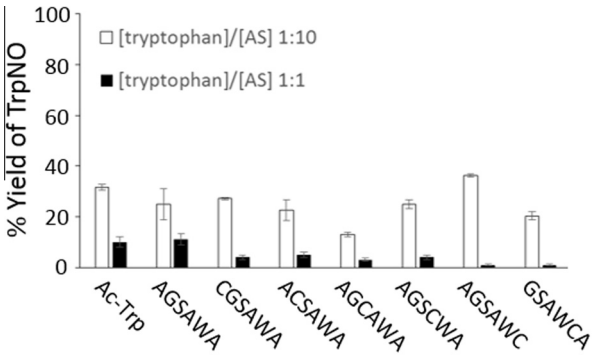

Figure 2. Percent yield of *N*-nitrosotryptophan (TrpNO) observed upon incubation of *N*-acetyl-L-tryptophan (Ac-Trp) or synthetic peptides with 10-fold excess ((□), [tryptophan] = 0.1 mM, [AS] = 1 mM) or equimolar ((■), [tryptophan] = 0.3 mM, [AS] = 0.3 mM) amounts of HNO-donor in 10 mM phosphate buffer with 50 μM DTPA (pH 7.4) at 37 °C for 30 min. The percent yield of TrpNO was determined by UV–visible spectroscopy with respect to the amount of tryptophan in the control samples (SEM ±6%, $n \geqslant 2$).

donor, 2-bromo-*N*-hydroxybenzenesulfonamide (2-BrPA)[15] under the same conditions (data not shown). These results are consistent with previous reports suggesting the formation of HNO-derived TrpNO,[12,13] and demonstrate that this modification is not specific to AS, but also takes place with other HNO-donors.

To assess the feasibility of an HNO-derived tryptophan modification in the vicinity of a free thiol, we treated the peptides containing both a tryptophan and a cysteine residue with different

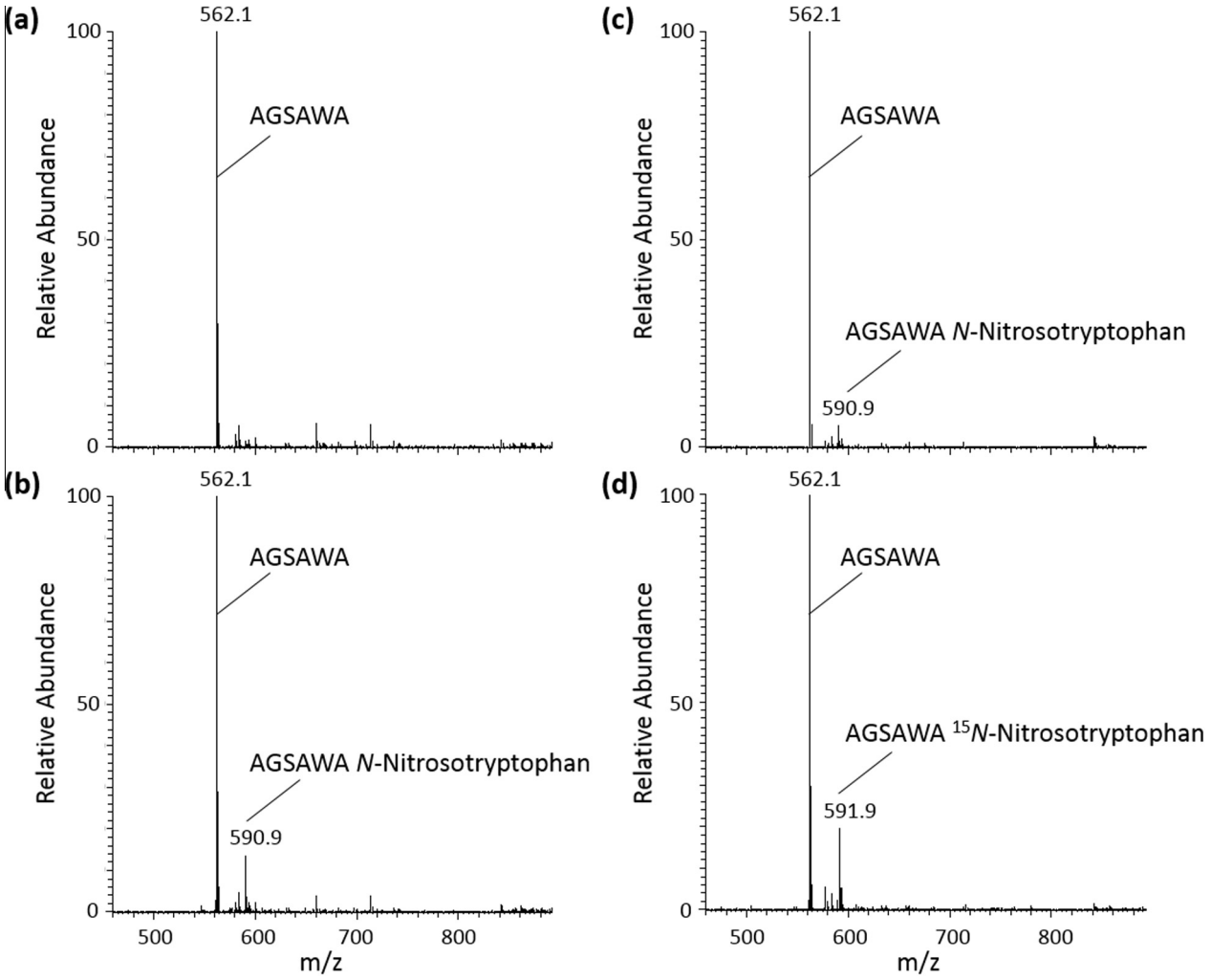

Figure 1. Selected region of ESI-MS spectra showing AGSAWA (0.3 mM) (a) untreated or treated with (b) 1 mM AS, (c) 1 mM 2-MSPA, and (d) 1 mM ^{15}N-2-MSPA in 10 mM phosphate buffer with 50 μM DTPA (pH 7.4) at 37 °C for 30 min.