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Water soluble acyloxy nitroso compounds: HNO release and reactions with heme and thiol containing proteins

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ABSTRACT

Nitroxyl (HNO) has gained interest as a potential treatment of congestive heart failure through the ability of the HNO donor, Angeli's salt (AS), to evoke positive inotropic effects in canine cardiac muscle. The release of nitrite during decomposition limits the use of AS requiring other HNO sources. Acyloxy nitroso compounds liberate HNO and small amounts of nitrite upon hydrolysis and the synthesis of the water-soluble 4-nitrosotetrahydro-2*H*-pyran-4-yl acetate and pivalate allows for pig liver esterase (PLE)-catalysis increasing the rate of decomposition and HNO release. The pivalate derivative does not release HNO, but the addition of PLE catalyzes hydrolysis ($t_{1/2}$ = 39 min) and HNO formation (65% after 30 min). In the presence of PLE, this compound converts metmyoglobin (MetMb) to iron nitrosyl Mb and oxyMb to metMb indicating that these compounds only react with heme proteins as HNO donors. The pivalate in the presence and the absence of PLE inhibits aldehyde dehydrogenase (ALDH) with IC_{50} values of 3.5 and 3.3 μM, respectively, in a time-dependent manner. Reversibility assays reveal reversible inhibition of ALDH in the absence of PLE and partially irreversible inhibition with PLE. Liquid chromatography–mass spectrometry (LC–MS) reveals formation of a disulfide upon incubation of an ALDH peptide without PLE and a mixture of disulfide and sulfinamide in the presence of PLE. A dehydroalanine residue forms upon incubation of this peptide with excess AS. These results identify acyloxy nitroso compounds as unique HNO donors capable of thiol modification through direct electrophilic reaction or HNO release.

1. Introduction

Nitroxyl (HNO/NO^-), is the one electron reduced relative of nitric oxide (NO), an important biological signaling agent [1–5]. Nitroxyl has distinct biological properties compared to NO and is a potential treatment of myocardial reperfusion injury and congestive heart failure [6–8]. Nitroxyl avidly reacts with heme and thiol containing proteins and these reactions likely mediate its biological actions [8–11]. Nitroxyl must be generated from donors due to its fast dimerization to nitrous oxide (N_2O, $k = 8 \times 10^6\ M^{-1}\ s^{-1}$) and a limited number of donors exist [12–14]. Angeli's salt ($Na_2N_2O_3$, AS) is the most widely used HNO donor, but at physiological pH AS forms an equivalent of nitrite during decomposition [15], Nitrite reacts with all of the redox forms of hemoglobin and possesses its own biological profile, which must be accounted for in biological evaluations of HNO activity [15–19]. Other limitations of AS include the fast rate of decomposition ($k = 10^{-4}\ s^{-1}$) and difficulty in modifying the structure [14,15]. The ability of AS to elicit positive inotropic effects in canine cardiac muscle highlights the need for new HNO donors as potential new therapies [7,20].

Acyloxy nitroso compounds (**1**–**3**, Fig. 1) act as HNO donors through ester hydrolysis to give an unstable intermediate that decomposes to HNO without generation of nitrite in buffered conditions [21]. Varying the pH and the R group of the ester of these structures varies their stability, HNO donor activity and ability to relax pre-constricted rat aorta [22]. Compounds **1** and **2** only slowly hydrolyze and competitively react with other nucleophiles (thiolates) without HNO formation, but **3** hydrolyzes to HNO under all conditions [22]. While compounds **1**–**3** provide information concerning the reactivity and HNO release of acyloxy nitroso compounds, their lack of water solubility limits their use in biological systems. Incorporating oxygen into the ring of **1** and **2** gives **4** and **5**, and should greatly increase water solubility allowing for esterase mediated hydrolysis to increase the rate of decomposition (Fig. 1) [23].[1]

The increased water solubility of **4**–**5** and their structural similarity to *C*-nitroso compounds, which also react with thiols [24], allow evaluation with myoglobin (Mb), a heme protein, and aldehyde dehydrogenase (ALDH), a thiol containing protein, known HNO targets in the presence of pig liver esterase (PLE) to enhance HNO release [11,25,26]. Our results show that acyloxy nitroso compounds can act

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[1] During this work, the following patent appeared describing the preparation of **4** and **5**.

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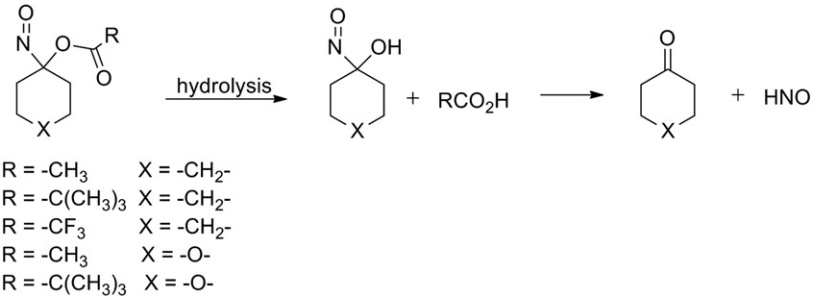

Fig. 1. Hydrolytic HNO release from **1**–**5**.

as efficient HNO donors in the presence of PLE, which may be useful in further probing HNO biology.

2. Experimental

2.1. General

All chemicals were purchased from Sigma Aldrich Chemical Company and used as received. AS and ^{15}N-AS were prepared as described, and stored dry at −20 °C [27]. Analytical TLC was performed on silica gel plates with C-4 Spectroline 254 indicator. Visualization was accomplished with UV light. Solvents for extraction and purification were of technical grade and used as received. Liquid chromatography–mass spectrometry (LC–MS) solvents were HPLC grade. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker Avance 300 MHz NMR spectrometer. Chemical shifts are given in ppm (δ); multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet). Yeast ALDH (E.C. 1.2.1.5) was purchased from Sigma Aldrich Chemical Company. UV–vis spectrometry was performed on a Cary 100 Bio UV–visible (UV–vis) spectrophotometer (Varian, Walnut Creek, CA). IC_{50}s were obtained by fitting concentration-dependent percent of control data non-linear curve fitting using sigmoidal curve with standard slope algorithm using GraphPad Prism software. ALDH peptide A286-R307 (AIDWIAAGIFYLNSGQNCTANSR) was purchased from Peptide 2.0 (Chantilly, VA) with >98% purity. High-resolution mass spectra were obtained using a Thermo Scientific LTQ Orbitrap mass spectrometer equipped with a heated electrospray ionization source operated in positive ion mode.

2.2. Synthetic procedures

2.2.1. 4-Nitrosotetrahydro-2H-pyran-4-yl Acetate (**4**)

Ultraviolet–visible (UV–vis) (PBS) λ_{max} = 660 nm, ε = 20.7 M^{-1} cm^{-1}, ^{1}H NMR (300 MHz, benzene-d_6) δ 1.5 (dd, J = 2.5, 14.0 Hz 2H), 1.7 (s, 3H), 1.9 (ddd, J = 5.0, 11.5 Hz, 2H), 3.3 (td, J = 2.7, 11.6 Hz, 2H), 3.6 (ddd, J = 4.6, 11.8 Hz, 2H), ^{13}C NMR (75 MHz, benzene-d_6) δ 21.8, 31.3, 64.2, 120.2, 167.6.

2.2.2. 4-Nitrosotetrahydro-2H-pyran-4-yl Pivalate (**5**) [22]

UV–vis (DMF:PBS, 1:29) λ_{max} = 660 nm, ε = 20.7 M^{-1} cm^{-1}, ^{1}H NMR (300 MHz, benzene-d_6) δ 1.2 (s, 9H), 1.5 (dd, J = 2.5, 14.0 Hz, 2 H), 1.9 (ddd, J = 5.0, 11.5 Hz, 2 H), 3.3 (td, J = 2.4, 11.5 Hz, 2H), 3.6 (ddd, J = 4.8, 11.7 Hz, 2H), ^{13}C NMR (75 MHz, benzene-d_6) δ 28.3, 31.1, 40.2, 64.3, 119.6, 175.0.

2.3. UV–vis spectroscopy assay for the decomposition of **4** and **5** [21]

A solution of phosphate buffered saline (PBS, 100 mM, pH 7.4) was added to a solution of **4** (5 μmol) in PBS (100 mM, pH 7.4, 100 μL) or **5** (5 μmol) in anhydrous dimethyl formamide (DMF) (50 μL) to give a total volume of 1.5 mL at 37 °C in a sealed cuvette. UV–vis spectra were taken every 5 min for 6 h at 660 nm. Similar experiments were performed using a solution of PLE (9 U/μmol) in PBS (100 mM, pH 7.4).

2.4. Gas chromatographic N_2O analysis [28]

For headspace analysis, a solution of **4** or **5** (0.08 μmol) in anhydrous DMF (8 μL) followed by PBS (10 mM, pH 7.4, 0.8 mL) was injected into a 10 mL round bottom flask sealed with a rubber septum and flushed with inert gas. To some samples PLE (9 U/μmol) and GSH (1.60 μmol) were added, the sample was incubated at 37 °C, and at desired timepoints headspace aliquots (100 μL) were injected via a gas-tight syringe onto a 7890 A Agilent Technologies gas chromatograph equipped with a micro-electron capture detector and a 30 m × 0.32 m (25 μm) HP-MOLSIV capillary column. The oven was operated at 200 °C for the duration of the run (4.5 min). The inlet was held at 250 °C and run in split mode (split ratio 1:1) with a total flow (N_2 as carrier gas) of 4 mL/min and a pressure of 37.9 psi. The micro electron capture detector (μECD) was held at 325 °C with a makeup flow (N_2) of 5 mL/min. The retention time of nitrous oxide was 3.4 min, and yields were calculated based on a standard curve for nitrous oxide (Matheson Tri-Gas).

2.5. Chemiluminescence NO, NO_2^- assay [21]

A solution of **4** or **5** (0.08 μmol) in anhydrous DMF (8 μL) followed by PBS (10 mM, pH 7.4, 0.8 mL) was injected into a 10 mL round bottom flask sealed with a rubber septum and flushed with inert gas. To some samples PLE (9 U/μmol) was added and the sample was incubated at 37 °C, and at desired timepoints, aliquots (200 μL headspace, NO or 5 μL reaction mixture, NO_2^-) were injected into the reaction vessel of a Sievers 280 NOA chemiluminescence detector. This apparatus detects nitrite by reduction of nitrite to NO, followed by chemiluminescence NO detection (the reaction vessel contained 1% NaI in glacial acetic acid). The yields were calculated based on a standard curve for nitrite.

2.6. UV–vis assay of Mb with 5

2.6.1. UV–vis assay of ferrous nitrosyl complex from the reaction of **5** with metmyoglobin (metMb)

Under degassed conditions, a solution of **5** (2.58 μmol) in anhydrous DMF (25.8 μL) was added to a solution of metMb (0.129 μmol) in PBS (100 mM, pH 7.4, 1.47 mL) at 37 °C in a sealed cuvette. In the reactions that contained PLE (9 U/μmol, 85 U/mL in PBS, 100 mM, pH 7.4), it was added directly before UV–vis measurements began. UV–vis spectra were taken every 1.0 min for 4 h, and the disappearance of metMb was monitored by the decrease in absorbance at 502 and 630 nm, while the appearance of Mb–NO was monitored by the increase in absorbance at 542 and 583 nm [26].