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Nitric oxide generation from *S*-nitrosoglutathione: New activity of indium and a survey of metal ion effects

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A B S T R A C T

S-Nitrosothiols (RSNOs) such as *S*-nitrosoglutathione (GSNO) are known to produce nitric oxide (NO) through thermal, photolytic, and metal ion-promoted pathways, which has led to their increasing use as exogenous sources of therapeutic NO. Despite the burgeoning NO release applications for RSNOs, their susceptibility to metal-promoted decomposition has rarely been examined in a uniform manner through the specific measurement of NO release. In this study, the ability of various transition and post-transition metal ions to promote NO release from GSNO was surveyed by chemiluminescence-based NO detection. Substantial NO formation (> 10-fold increase relative to GSNO baseline) was detected after the addition of Cu^{2+}, Au^{3+}, Pd^{2+}, Pt^{2+}, and V^{3+}. Modest increases were observed in the cases of Co^{2+}, Hf^{4+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Hg^{2+}, Ni^{2+}, Ag^{+}, Sn^{2+}, and Zr^{4+}, while no effect was evident for Al^{3+}, Cr^{3+}, Pb^{2+}, Sc^{3+}, and Zn^{2+}. It was further observed that In^{+} compounds initiate the apparent NO-forming decomposition of GSNO, while In^{0} and In^{3+} are inactive, indicating that In^{+} exerts a previously unknown effect on GSNO.

1. Introduction

S-Nitrosothiols (RSNOs) occur naturally in human biochemistry, with apparent blood concentrations typically reported to fall within the nM to μM range [1]. Endogenous RSNOs are generally derived from *S*-nitrosation of cysteine residues in proteins such as *S*-nitrosoalbumin or low-molecular weight peptides such as *S*-nitrosoglutathione (GSNO). While the physiological production and function of RSNOs remains the subject of discussion, these unique compounds have attracted considerable interest from a pharmacological perspective as sources of the diatomic radical nitric oxide (NO). NO bioactivity is currently recognized to include vasodilation, maintenance of the anticoagulative properties of the vasculature, neurotransmission, wound-healing, and the immune response [2,3]. RSNOs are typically observed to possess limited solution-phase stability and decompose to form NO and disulfide through thermal, photolytic, and metal ion-promoted pathways [4]. The most striking example of the latter pathway is observed in the case of Cu^{+}, which has been demonstrated to readily catalyze RSNO decomposition [5]. Similarly, Fe^{2+} has been found to induce the NO-forming decomposition of RSNOs in a limited manner [6]. The ability of RSNOs to produce NO in this fashion has led the biomaterials community to routinely propose the use of various metal or metal ion-based biointerfacial materials to generate therapeutic NO directly from endogenous sources, primarily through contact with blood [7–10]. Despite considerable interest in the use of metals or metal ions to trigger NO formation from RSNOs, few studies have specifically surveyed the ability of common transition and post-transition metal ions to produce this effect. Prior reports often confined their analysis to a limited range of period 4 d-block metals (e.g., Cr, Mn, Fe, Co, Ni, Cu, Zn) and utilized techniques such as UV–Vis absorption spectroscopy that monitor RSNO decomposition rather than NO formation [2,4,11]. In this study, NO-selective chemiluminescence-based detection was used to evaluate the effect of various metal ions on GSNO, a model RSNO with well-characterized chemical behavior.

2. Materials and methods

2.1. Materials

Cobalt(II) chloride hexahydrate (> 98%), lead(II) nitrate (≥99.0%), and nickel(II) nitrate hexahydrate (99%) were purchased from Acros Organics (Morris Plains, NJ, USA). Chromium(III) chloride hexahydrate (98%), indium(I) chloride (99.995%), indium(III) chloride (99.999%), indium(I) iodide (99.998%), indium powder (−325 mesh,

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99.999%), mercury(II) acetate (98%), silver(I) nitrate (99.995%), tin (II) chloride dihydrate (98%), vanadium(III) chloride (99%), and zirconium dichloride oxide hydrate (99.9%) were purchased from Alfa Aesar (Ward Hill, MA, USA). Copper(II) chloride dihydrate (99%) was purchased from EMD Chemicals (Gibbstown, NJ, USA). Iron(III) chloride hexahydrate (≥97%) was purchased from Mallinckrodt (St. Louis, MO, USA). Iron(II) chloride tetrahydrate (> 99%), manganese (II) chloride tetrahydrate (> 99%), and zinc(II) chloride (> 97%) were purchased from Fisher Scientific (Waltham, MA, USA). *N*-Acetyl-D-penicillamine (≥99.0%), aluminum nitrate nonahydrate (≥98%), gold (III) chloride trihydrate (≥49.0% Au), hafnium(IV) chloride tetrahydrofuran complex (98%), indium(II) chloride (99.9%), neocuproine (≥98%), scandium(III) triflate (99%), sodium nitrite (99.5%), sodium tetrachloropalladate(II) (98%), and sodium tetrachloroplatinate(II) hydrate (≤49.8% Pt) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Nalgene surfactant-free cellulose acetate syringe filters (0.2 μm) were obtained from Thermo Scientific (Waltham, MA, USA). Reduced glutathione was purchased from VWR (Radnor, PA, USA). All chemicals were used as received. Hygroscopic compounds were stored in a desiccator. Ultra-high-purity (UHP) nitrogen (N_2) and oxygen (O_2) gases were purchased from Airgas (Denver, CO, USA). Nitric oxide (NO; 43.6 ppm NO, balance N_2) calibration gas was supplied by Air Liquide (Houston, TX, USA). Deionized water (18.2 MΩ·cm) was supplied by a Millipore Direct-Q water purification system (EMD Millipore, Billerica, MA).

2.2. Analytical methodology

General analytical techniques. Nuclear magnetic resonance (NMR) spectra were acquired using a Bruker Avance Neo 400 NMR spectrometer (Billerica, MA, USA). Ultraviolet–visible (UV–Vis) spectroscopy was performed using a Nicolet Evolution 300 spectrophotometer (Thermo Electron Corp., Madison, WI, USA) and quartz cuvettes. Mass spectra were acquired using an Agilent 6224 time-of-flight mass spectrometer (TOF-MS) equipped with a dual electrospray ion source in negative ion mode.

Measurement of NO production. Nitric oxide (NO) release from *S*-nitrosoglutathione (GSNO) was quantified using chemiluminescence-based Sievers NO analyzers (NOA 280i) (Zysense, Weddington, NC, USA). The instruments were operated using UHP N_2 gas to transport NO from a custom external measurement vessel (200 mL min^{-1} flow rate) to the internal reaction cell (4–7 Torr, −12.0 °C) for quantification by chemiluminescent reaction with ozone (produced internally from O_2 gas supplied at 5.8–6.2 psig). Prior to use, the instruments were calibrated using UHP N_2 (0 ppm NO) and 43.6 ppm NO/N_2. During NO production experiments, data was recorded as a function of time (s) in ppb/ppm values that were subsequently converted to moles of NO using instrument-specific calibration constants (mol NO ppb^{-1} s^{-1}). These calibration constants were obtained from the reduction of known quantities of sodium nitrite to NO by potassium iodide in dilute sulfuric acid, and were verified by the NO-forming decomposition of known quantities of GSNO in the presence of copper(II) chloride. In standard experiments, soluble compounds were dissolved in Millipore water (18.2 MΩ·cm) and transferred to the external measurement vessel, where N_2 was bubbled through the solution to remove dissolved O_2. GSNO (dissolved in Millipore water) was then added to the vessel to produce an initial concentration of 2.50×10^{-4} M GSNO (1.25×10^{-6} mol RSNO) and 2.50×10^{-5} M of the soluble compound (1.25×10^{-7} mol overall) in a total solution volume of 5.00 mL. Real-time production of NO was then recorded at measurement intervals of 1 s for either 0.5 or 1.0 h. Continuous bubbling of N_2 was used to ensure the rapid displacement of NO from the solution. In cases where the element or compound was insoluble, 1×10^{-5} mol of the substance was added to the measurement vessel as a solid and suspended in a total solution volume of 5.00 mL of Millipore water containing 2.50×10^{-4} M GSNO (1.25×10^{-6} mol GSNO). Select experiments were repeated at a lower GSNO concentration of 2.50×10^{-5} M, following an otherwise identical protocol. Reactions were unbuffered to avoid effects from buffer solutes, and the pH was generally governed by the presence of GSNO at 2.50×10^{-4} M (ca. pH 4) or 2.50×10^{-5} (ca. pH 5) concentrations. Between experiments, all glassware was treated with a saturated aqueous solution of EDTA-Na_2 and extensively washed with Millipore water.

2.3. Synthesis of S-nitrosoglutathione (GSNO)

In a typical synthesis, the compound was prepared following an adaptation of the method reported by Hart [12]. Glutathione (GSH; 1.53 g, 5 mmol) was suspended in 4 mL of Millipore water and dissolved by the addition of 2.5 mL of 2 M hydrochloric acid. This mixture was subsequently cooled to 0 °C using an ice bath. Separately, sodium nitrite (0.345 g, 5 mmol) was dissolved in 4 mL of Millipore water and added to the stirring solution of GSH. The mixture rapidly developed a red-pink color that was accompanied by the formation of a pink precipitate, and was stirred in the absence of light for 40 min. After this period, 10 mL of acetone was added to the reaction to promote further precipitation of GSNO. The mixture was allowed to stir for 10 min, then the precipitate was isolated by vacuum filtration. The precipitate was washed with 3 × 5 mL of ice-cold Millipore water, 3 × 10 mL of acetone, and 3 × 10 mL of diethyl ether. The precipitate was placed under vacuum for several hours to remove residual solvent and yielded GSNO (1.28 g, 76%) as a fine pink powder. ^{1}H NMR (400 MHz, D_2O): δ 4.64 (t, cysteinyl -CH-, 1H), 4.17-3.90 (m, cysteinyl -CH_2-, 2H), 3.92 (s, glycinyl -CH_2-, 2H), 3.76 (t, glutamyl -CH-, 1H), 2.41 (t, glutamyl -CH_2CO-, 2H), 2.16–2.00 ppm (m, glutamyl -CH_2-, 2H). UV–Vis (H_2O): λ 335 (RSNO π → π*), 545 nm (RSNO n → π*) (Supplementary Figs. 1 and 2).

2.4. Data reporting and statistical analysis

Data reported as mean ± standard deviation of a minimum of three replicate experiments. The ability of tested metals to promote elevated NO release from GSNO or *S*-nitroso-*N*-acetyl-D-penicillamine (SNAP) was determined by comparison of total NO release (mol) over the measurement period (0.5–1.0 h) with the baseline NO release of GSNO or SNAP in the absence of metal ions, using the two-tailed Student's t-test ($p < 0.05$).

3. Results and discussion

Previous studies concerning the ability of metal ions to interact with RSNOs have utilized varying substrates and analytical approaches that have occasionally resulted in conflicting reports (Supplementary Fig. 3). The use of a uniform experimental approach offers the clear benefit of permitting direct comparisons of relative metal ion activity under a specific set of measurement conditions. In this work, we sought to directly measure the metal-promoted release of gaseous NO from an aqueous 25 or 250 μM RSNO solution using NO-selective chemiluminescence-based detection. This technique requires anaerobic conditions to avoid consumption of NO prior to detection, which also prevents rapid oxidation of sensitive metal species by O_2. GSNO represented a logical choice of RSNO substrate due to its biological relevance, well-characterized chemical behavior, and stability. We found that the real-time measurement of NO formation over 0.5 h was sufficient to identify cases in which the addition of particular metal compounds resulted in elevated NO production relative to control experiments. Selected experiments were continued for 1 h to permit examination of NO release over a longer duration. All metal ions were evaluated at a GSNO concentration of 250 μM, and those species that promoted substantial NO production in the absence of previously hypothesized nitrous acid (HNO_2)-forming activity were further tested at 25 μM. The results of this survey are summarized in Table 1 as cumulative NO production, and are compared with the typical quantity of NO released from GSNO

Table 1
Metal-promoted nitric oxide release from *S*-nitrosoglutathione.

Compound[a]	Time (h)	NO (nmol)[b]	NO (nmol)[b,d]
		250 μM	25 μM
None (control)	0.5	0.57 ± 0.14	*nt*
None (control)	1.0	0.81 ± 0.11	0.16 ± 0.15
$Al(NO_3)_3$	0.5	0.78 ± 0.06	*nt*
$CrCl_3$	0.5	0.57 ± 0.10	*nt*
$CoCl_2$	0.5	1.43 ± 0.19[c]	*nt*
$CuCl_2$	1.0	92.4 ± 2.2[c]	120 ± 5
$AuCl_3$	1.0	15.2 ± 0.2[c]	3.67 ± 0.69
$HfCl_4$	1.0	3.40 ± 0.30[c]	*nt*
In (metallic)	0.5	0.73 ± 0.07	*nt*
InCl	1.0	57.9 ± 6.7[c]	18.1 ± 0.7
$InCl_2$	1.0	26.1 ± 3.0[c]	15.1 ± 3.3
$InCl_3$	0.5	0.63 ± 0.07	*nt*
InI	1.0	15.7 ± 0.7[c]	28.9 ± 4.5
$FeCl_2$	1.0	4.61 ± 0.28[c]	0.87 ± 0.11
$FeCl_3$	0.5	1.58 ± 0.11[c]	*nt*
$Pb(NO_3)_2$	0.5	0.59 ± 0.04	*nt*
$MnCl_2$	0.5	0.99 ± 0.07[c]	*nt*
$Hg(CH_3CO_2)_2$	1.0	6.52 ± 0.12[c]	*nt*
$Ni(NO_3)_2$	0.5	0.82 ± 0.06[c]	*nt*
$Sc(SO_3CF_3)_3$	0.5	0.70 ± 0.10	*nt*
$AgNO_3$	0.5	1.12 ± 0.05[c]	*nt*
Na_2PdCl_4	1.0	19.8 ± 1.2[c]	6.40 ± 0.18
Na_2PtCl_4	1.0	14.0 ± 0.6[c]	4.18 ± 0.38
$SnCl_2$	1.0	4.39 ± 0.27[c]	*nt*
VCl_3	1.0	20.6 ± 0.3[c]	*nt*
$ZnCl_2$	0.5	0.75 ± 0.04	*nt*
$ZrOCl_2$	1.0	6.48 ± 0.12[c]	0.78 ± 0.04

[a] Soluble compounds dissolved at a concentration of 25 μM. Insoluble compounds added as solids (10 μmol).
[b] Cumulative NO release over the indicated time. Data reported as mean ± SD of $n \geq 3$ replicate measurements.
[c] Cumulative NO release significantly different ($p < 0.05$) from control experiment by two-tailed Student's t-test.
[d] Metal ions not tested at 25 μM GSNO labeled *nt* for clarity.

at 25 °C in the absence of light. To interpret the potential for other trace metals to influence the experimental results, ICP-AES was used to determine the concentration of possible contaminants. Due to the potency of Cu species in liberating NO from GSNO, the presence of this metal as a trace contaminant in other experiments is given particular attention in tabular form and presented in Supplementary Table 1. We observed that the addition of 25 μM Cu^{2+} to a 250 μM anaerobic solution of GSNO predictably induces a greater than 100-fold increase in the total quantity of NO released over the 1 h measurement period. As described elsewhere, the reaction is believed to involve reduction of Cu^{2+} to Cu^{+} by trace thiol, and it is this reduced metal species that initiates catalytic decomposition of the RSNO through a mechanism that has yet to be fully elucidated [5]. At a lower GSNO concentration of 25 μM, the efficiency of the reaction is significantly improved, resulting in both a greater 1 h NO yield (48% compared with 7.4%), and a higher cumulative NO recovery (120 ± 5 nmol compared with 92.4 ± 2.2 nmol) (Supplementary Fig. 4). This outcome can be explained by a decrease in the ratio of dissolved Cu to substrate at the higher GSNO concentration, as well as binding of Cu^{+}/Cu^{2+} by GSNO or disulfide that leads to inhibition of catalytic activity [13,14]. Other processes such as autocatalysis and radical recombination are also hypothesized to influence RSNO decomposition in a concentration-dependent manner [15]. The susceptibility of GSNO solutions to decomposition after the addition of Cu^{2+} is decreased under aerobic conditions, where oxidation of catalytically-active Cu^{+} inhibits the reaction [5]. In contrast with prior reports from Williams et al. that describe Fe^{3+} as inert with respect to RSNO decomposition, we find that both Fe^{2+} (Supplementary Fig. 5) and Fe^{3+} detectably elevated the rate of NO production from GSNO under deoxygenated conditions. These effects were far less dramatic than in the case of Cu, and the apparent activity of Fe^{3+} may simply arise from reduction to Fe^{2+} by trace thiol in a manner analogous to Cu^{2+}/Cu^{+} [6]. Among d- and p-block metals, only Cu and Fe ions are widely accepted to directly produce NO via decomposition of RSNOs. Krężel et al. reported that Ni^{2+} also decomposes GSNO in a concentration-dependent manner [16]. McCarthy et al. identified similar behavior in the case of Ni^{2+}, and expanded this activity to Co^{2+} and Zn^{2+}, although the outcome with these examples contradicted previous publications and has remained largely uncorroborated [17]. Our results support the inactivity of Cr^{3+} and Zn^{2+}, although a subtle increase in NO formation from GSNO was detected in the presence of both Co^{2+} and Ni^{2+}. A similarly marginal increase in NO production was observed after the addition of Mn^{2+}, which has been frequently characterized as inactive by Williams and others. Interestingly, it was also observed by Krężel et al. that equimolar Zn^{2+} (and to a lesser extent, Cd^{2+}) was able to *decrease* the rate of GSNO decomposition, which was hypothesized to result from binding of trace thiol that is otherwise a participant in the decomposition pathway [16]. We found that the addition of Al^{3+}, Pb^{2+}, or Sc^{3+} does not result in statistically significant acceleration of the NO-forming decomposition of GSNO, while Hf^{4+}, Sn^{2+}, and Zr^{4+} produce effects of a magnitude similar to or greater than the activity of Fe^{2+}. The apparent activity of V^{3+} is perhaps explained by the presence of trace Cu, which is detected by ICP-AES at a concentration of 10^{-7} M. However, GSNO itself contains a detectable quantity of trace Cu that is greater than the contribution from VCl_3 and insufficient to induce significant decomposition.

Alternatively, the known ability of V^{3+} to reduce NO_2^{-} to NO may indicate that HNO_2 liberated by RSNO hydrolysis is the source of the detected NO [18]. In general, our data support observations from Ewing and Janero, who found that V^{3+} forms NO from multiple RSNOs [19]. The ability of noble metals to interact with RSNOs has been the subject of several earlier studies. For example, Jia et al. described the use of Au^{0} nanoparticles to induce NO formation from SNAP [20]. We observed that Au^{3+}, Pd^{2+}, and Pt^{2+} induce NO formation from GSNO, contrasting with at least one prior report that found no effect in the case of Pt^{2+} and SNAP [17]. The reaction of Hg^{2+} with RSNOs facilitates their rapid hydrolysis to nitrous acid (HNO_2), a process that forms the basis of the Saville assay [21]. It has been observed by Swift and Williams that Ag^{+} is similarly able to promote the HNO_2^{-} forming hydrolysis of RSNOs, albeit at a reduced rate [22]. While we observed minor activity with both Hg^{2+} and Ag^{+}, the detection of NO is likely to be a consequence of HNO_2 formation and subsequent decomposition to NO [23].

No clear pattern emerges from the data that permits prediction of active metals, especially given the probability that multiple mechanistic pathways lead to the formation of NO (Fig. 1). Regardless of whether NO formation occurs directly or through decomposition of HNO_2, it may be reasonable to hypothesize that the activity of many thiophilic metals is indicative of a conserved elementary step: coordination of sulfur to the metal species. The physical and chemical properties of RSNOs have frequently been rationalized through a model that

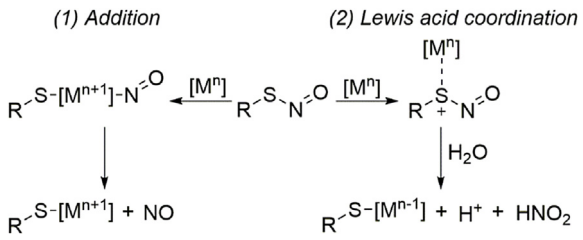

Fig. 1. Speculative metal-promoted decomposition pathways of *S*-nitrosothiols. In the first pathway, the addition of a metal to the S-N bond precedes the production of NO in a manner similar to the behavior of certain copper [26], osmium, or ruthenium complexes [27]. In the second pathway, coordination of a Lewis acidic metal facilitates hydrolysis to HNO_2, which can subsequently decompose to form detectable NO in the absence of O_2 [21].

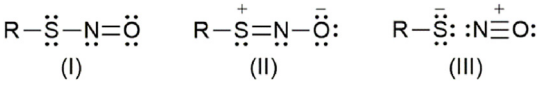

Fig. 2. Hypothesized resonance structures of *S*-nitrosothiols.

proposes three distinct resonance structures (Fig. 2) [24]. In this model, coordination of the sulfur atom to a Lewis acid is anticipated to disfavor structure II, which weakens the S-N bond and renders it susceptible to hydrolytic cleavage (e.g., Hg^{2+} in the Saville assay). The thiophilicity of a given metal species may contribute to the likelihood of this outcome. In contrast, coordination by nitrogen and oxygen can stabilize RSNOs [24,25]. Thiophilicity may remain an important factor in the addition or coordination of RSNOs to metal centers, as in the case of certain copper [26] complexes, as well as ruthenium and osmium metalloporphyrins [27]. In the latter examples, initial coordination of sulfur to the metal center is proposed to precede formal addition. While the formation of these complexes is not uniformly productive with respect to NO, RSNO decomposition by Cu^{+} has been hypothesized to proceed through cyclic metal chelates or addition complexes that involve similar sulfur-metal interactions [4,26]. The notion that favorable sulfur-metal coordination is a critical step in the formation of NO or HNO_2 is apparently contradicted by the activity of strongly oxophilic Lewis acids like Hf^{4+}, V^{3+}, and Zr^{4+} [28]. Moreover, this principle correctly predicts the behavior of certain p-block metals (Sn^{2+}) but not others (Pb^{2+}). It is clear that additional factors beyond thiophilicity alone influence the ability of metal ions to promote NO or HNO_2 formation from GSNO.

Curiously, many species that could reasonably be expected to induce the NO-forming decomposition of RSNOs have yet to be investigated. For instance, exploration of the effect of In on RSNOs may be justified by certain chemical similarities between In^{+} and Cu^{+}, which are *superficially* related by the "Knight's Move" pattern described by Laing [29,30]. Inspired by this admittedly tenuous connection, we performed experiments in which 25 or 250 μM GSNO in water was exposed to solid InCl (10 μmol), resulting in the production of NO as determined by chemiluminescence-based detection (Fig. 3a, Supplementary Fig. 6). With larger quantities of InCl (≥40 μmol), we found that it was possible to variably recover as much as 50% of theoretical NO (based on the initial amount of GSNO). In these cases, another addition of InCl late in the reaction was not found to produce a significant increase in the rate of NO production or yield. The putative mixed-valence dihalide $InCl_2$ was similarly able to promote NO release from GSNO, presumably through contribution of In^{+} rather than activity from a discrete In^{2+} oxidation state (Fig. 3b). It is notable that compounds of In^{+} exhibit limited aqueous stability and rapidly disproportionate to In^{0} and In^{3+}, resulting in the formation of secondary species that could conceivably be responsible for the decomposition of GSNO [31]. In our study, this disproportionation resulted in the development of grey-white In^{0} from the previously yellow InCl sample

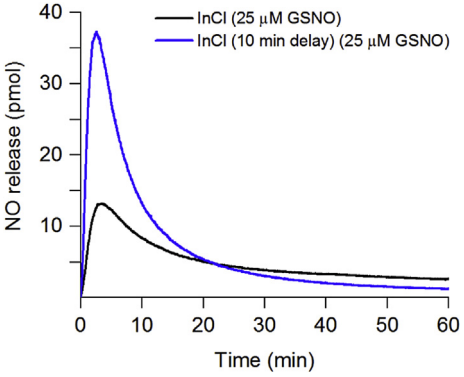

Fig. 4. NO production from 25 μM *S*-nitrosoglutathione (GSNO) in the presence of InCl under typical experimental conditions (black) and after immersion in water for 10 min prior to the addition of GSNO (blue). Plotted data are the average of n ≥ 3 replicate experiments.

and the release of In ions into solution at a near-mM concentration. In subsequent experiments, we failed to observe significant NO production from GSNO in the presence of either In^{0} or In^{3+} (added as metallic powder or water-soluble $InCl_3$), which was taken as an indication that these oxidation states do not independently contribute to the activity of InCl. The NO-forming reaction was temporarily slowed (but not fully arrested) by metal ion chelators, including EDTA-Na_2 and Cu^{+}-selective neocuproine. However, the formation of a distinct neocuproine-Cu^{+} complex (λ_{max} *ca.* 450 nm) [5] was not observed using UV–Vis spectroscopy, nor was the presence of dissolved Cu detected by ICP-AES. Spectroscopic changes occurred exclusively below 400 nm and overlapped with absorbance bands from neocuproine itself. Moreover, the reaction was never observed to result in quantitative recovery of theoretical NO, as is typically possible in Cu-catalyzed decomposition of RSNOs. Efficiency of the reaction was improved by 38% (25.0 ± 3.2 nmol cumulative NO release) after immersion of InCl in water for 10 min prior to addition of GSNO (25 μM final concentration) (Fig. 4). Even after immersion in water for 3 days prior to addition of the GSNO substrate, InCl maintained significant activity. Immersion of InCl in water for 10 min followed by passage of the solution through a sub-micron filter produced a clear filtrate that remained capable of initiating NO formation from GSNO. Permitting the filtrate to stand for 1 day prior to addition of the substrate substantially reduced the NO-forming activity, and it was arrested altogether by EDTA-Na_2. In general, these observations are consistent with a homogeneous active species (most likely In^{+}) that has a limited lifetime in water before inactivation, rather than a heterogeneous process that is localized to the insoluble solid. Based on the improved reaction efficiency after 10 min of immersion in water prior to substrate addition, it may be reasonable to surmise that this period is associated with some degree of accumulation of the active species in solution. To evaluate the ability of an

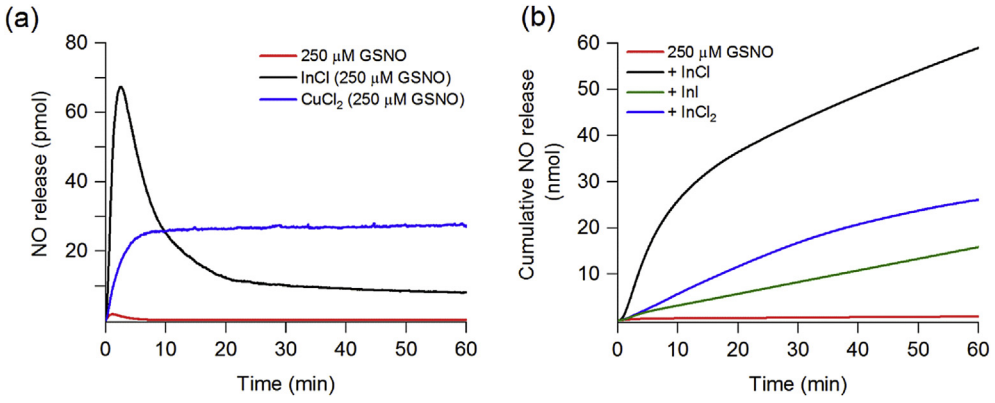

Fig. 3. (a) NO production from 250 μM *S*-nitrosoglutathione (GSNO) in the presence of InCl (black) or $CuCl_2$ (blue) at 25 °C . Baseline NO release from GSNO (red) is provided for comparison. (b) Cumulative NO release from 250 μM *S*-nitrosoglutathione (GSNO) in the presence of InCl (black), InI (green), and $InCl_2$ (blue). Traces for metallic indium and $InCl_3$ are indistinguishable from GSNO alone and are not shown. Plotted data are the average of n ≥ 3 replicate experiments.

additional In^+ halide species to promote NO release from GSNO, the effect of InI was examined following an identical measurement protocol. We observed that the ability of In^+ to initiate the NO-forming decomposition of GSNO was also exhibited in the monoiodide form, which produced NO at a steady rate over the 1 h measurement period. This behavior contrasts with the apparent exponential decay kinetics observed for the release of NO from GSNO in the presence of InCl, and is attributable to the distinct morphology of InCl powder compared to InI beads, which may result in differences in the availability of the hypothesized In^+ active species. When 25 μM SNAP was used in place of GSNO, InCl was similarly able to induce production of NO (34.1 ± 2.5 nmol), indicating that the scope of the reaction includes both primary (GSNO) and tertiary (SNAP) RSNO substrates (Supplementary Figs. 7–8).

Regardless of the In^+ source, NO is undoubtedly formed as a product of its interaction with RSNOs, yet it remains unclear if this occurs through direct generation of NO (as in the case of Cu^+) or through formation of HNO_2 that subsequently decomposes to NO. In either event, the yield of NO is neither quantitative nor significantly improved by a subsequent addition of InCl. This may suggest a complex overall reaction in which NO is not produced stoichiometrically from the RSNO substrate, perhaps supporting the intermediacy of HNO_2 or formation of other nitrogen oxide species. However, the comparatively poor Lewis acidity [31] of In^+ (particularly in comparison to inactive In^{3+}) indicates that HNO_2-forming hydrolysis is an unlikely pathway. Moreover, Lewis acid-promoted formation of HNO_2 presumably requires sufficient metal thiophilicity (e.g., Hg^{2+}) to trap thiolate and prevent reversal of the reaction. As an alternative hypothesis, NO production following oxidative addition of In^+ to the S-N bond may proceed in a manner analogous to the activity of certain Cu^+ complexes [26]. A mechanism of this type is generally more consistent with the known behavior of low-valent In, and various semi-stable In^+/In^{3+} thiolates have been electrochemically prepared that could serve as intermediates [32]. When characterized by ^1H NMR, the organic product of the InCl/GSNO reaction was unambiguously derived from the general tripeptide structure of glutathione, but was distinct from both oxidized and reduced glutathione (Supplementary Figs. 9–11). A control experiment performed without the addition of InCl resulted in significant retention of GSNO, as well as clear formation of the disulfide (Supplementary Fig. 12). In contrast, GSNO was no longer present following reaction with InCl. The significant downfield shift of the glutamyl α-proton (ca. 1 ppm) in the organic product supports reaction of the amine group (perhaps simply through protonation), yet a shift of this magnitude could not be independently achieved by dissolution of oxidized or reduced glutathione in 1 M DCl/D_2O. Mass spectrometry failed to identify a distinguishable organic product during reaction with InCl (Supplementary Figs. 13 and 14).

4. Conclusions

Our study indicates that a number of transition and post-transition metal ions are able to induce detectable NO release from GSNO, and that this activity includes a much broader variety of metal species than were previously identified. The established chemistry of RSNOs permits the prediction of two general reaction pathways: (a) the direct, NO-forming interaction of metal ions with RSNOs, and (b) hydrolysis of RSNOs to yield HNO_2, which subsequently decomposes to form a measurable gas-phase concentration of NO. It is reasonable to surmise that the latter pathway is significant exclusively in cases where the metal species possesses sufficient thiophilicity to inhibit the reverse reaction, or when such as a reversal is otherwise disfavored (i.e., when rapid removal or consumption of HNO_2 prevails). The majority of practical applications for metal ion-induced RSNO decomposition are currently achieved through the use of Cu^{2+}/Cu^+. Moreover, many fundamental studies of RSNO interactions with metal ions are limited to Cu^{2+}/Cu^+, Fe^{2+}, and a small selection of other transition metals, yet it is clear that other species have NO-forming effects that merit further examination. In particular, the previously unknown activity of In^+ is both unusual and distinctly confined to the low-valent form, in a manner that is potentially analogous to the behavior of Cu^+. The fact that In^+ compounds must be added as measurable quantities of solid limits our ability to assess the comparative activity of InCl/InI relative to soluble metal species. This inherent limitation is compounded by the propensity of In^+ compounds to disproportionate to In^0/In^{3+}. For these reasons, the precise concentration of the active species is unknown and it may be present in a significant excess over other tested species. At this stage, the stoichiometry associated with the reaction of In^+ with RSNOs is uncertain, and the exact nature of the products and their comparative distribution is similarly undetermined. Moreover, the practical use of In^+ species for NO generation from RSNOs is clearly limited by the considerations addressed earlier. Nevertheless, further study of this system may provide useful mechanistic insight into metal-promoted decomposition of RSNOs.

Declarations of interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.niox.2019.01.005.

References

[1] D. Giustarini, A. Milzani, I. Dalle-Donne, R. Rossi, Detection of S-nitrosothiols in biological fluids: a comparison among the most widely applied methodologies, J. Chromatogr. B: Anal. Technol. Biomed. Life Sci. 851 (2007) 124–139.
[2] A.R. Butler, D.L.H. Williams, The physiological role of nitric oxide, Chem. Soc. Rev. 22 (1993) 233–241.
[3] M.B. Witte, A. Barbul, Role of nitric oxide in wound repair, Am. J. Surg. 183 (2002) 406–412.
[4] D.L.H. Williams, The chemistry of S-nitrosothiols, Acc. Chem. Res. 32 (1999) 869–876.
[5] A.P. Dicks, H.R. Swift, D.L.H. Williams, A.R. Butler, H.H. Al-Sa'doni, B.G. Cox, Identification of Cu^+ as the effective reagent in nitric oxide formation from S-nitrosothiols (RSNO), J. Chem. Soc., Perkin Trans. 2 (1996) 481–487.
[6] A.F. Vanin, I.V. Malenkova, V.A. Serezhenkov, Iron catalyzes both decomposition and synthesis of S-nitrosothiols: optical and electron paramagnetic resonance studies, Nitric Oxide 1 (1997) 191–203.
[7] Y. Wo, E.J. Brisbois, R.H. Bartlett, M.E. Meyerhoff, Recent advances in thromboresistant and antimicrobial polymers for biomedical applications: just say yes to nitric oxide (NO), Biomater. Sci. 4 (2016) 1161–1183.
[8] B.K. Oh, M.E. Meyerhoff, Spontaneous catalytic generation of nitric oxide from *S*-nitrosothiols at the surface of polymer films doped with lipophilic copper(II) complex, J. Am. Chem. Soc. 125 (2003) 9552–9553.
[9] M.J. Neufeld, A. Lutzke, W.M. Jones, M.M. Reynolds, Nitric oxide generation from endogenous substrates using metal–organic frameworks: inclusion within poly (vinyl alcohol) membranes to investigate reactivity and therapeutic potential, ACS Appl. Mater. Interfaces 9 (2017) 35628–35641.
[10] T.C. Major, D.O. Brant, C.P. Burney, K.A. Amaoko, G.M. Annich, M.E. Meyerhoff, H. Handa, R.H. Bartlett, The hemocompatibility of a nitric oxide generating polymer that catalyzes S-nitrosothiol decomposition in an extracorporeal circulation model, Biomaterials 32 (2011) 5957–5969.
[11] J.S. Stamler, E.J. Toone, The decomposition of thionitrites, Curr. Opin. Chem. Biol. 6 (2002) 779–785.
[12] T.W. Hart, Some observations concerning the S-nitroso and S-phenylsulphonyl derivatives of L-cysteine and glutathione, Tetrahedron Lett. 26 (1985) 2013–2016.
[13] D.R. Noble, H.R. Swift, D.L.H. Williams, Nitric oxide release from S-nitrosoglutathione (GSNO), Chem. Commun. (1999) 2317–2318.
[14] D.R. Noble, D.L.H. Williams, Structure–reactivity studies of the Cu^{2+}-catalyzed decomposition of four *S*-nitrosothiols based around the *S*-nitrosocysteine/*S*-

nitrosoglutathione structures, Nitric Oxide 4 (2000) 392–398.
[15] M.G. de Oliveira, S.M. Shishido, A.B. Seabra, N.H. Morgon, Thermal stability of primary S-nitrosothiols: roles of autocatalysis and structural effects on the rate of nitric oxide release, J. Phys. Chem. 106 (2002) 8963–8970.
[16] A. Krężel, W. Bal, Contrasting effects of metal ions on *S*-nitrosoglutathione, related to coordination equilibria: GSNO decomposition assisted by Ni(II) vs stability increase in the presence of Zn(II) and Cd(II), Chem. Res. Toxicol. 17 (2004) 392–403.
[17] C.W. McCarthy, R.J. Guillory II, J. Goldman, M.C. Frost, Transition-metal-mediated release of nitric oxide (NO) from *S*-nitroso-*N*-acetyl-D-penicillamine (SNAP): potential applications for endogenous release of NO at the surface of stents via corrosion products, ACS Appl. Mater. Interfaces 8 (2016) 10128–10135.
[18] R.S. Braman, S.A. Hendrix, Nanogram nitrite and nitrate determination in environmental and biological materials by vanadium(III) reduction with chemiluminescence detection, Anal. Chem. 61 (1989) 2715–2718.
[19] J.F. Ewing, D.R. Janero, Specific *S*-nitrosothiol (thionitrite) quantification as solution nitrite after vanadium(III) reduction and ozone-chemiluminescent detection, Free Radic. Biol. Med. 25 (1998) 621–628.
[20] H. Jia, X. Han, Z. Li, Q. Tian, X. Miao, L. Du, Y. Liu, Gold nanoparticles-based catalysis for detection of S-nitrosothiols in blood serum, Talanta 85 (2011) 1871–1875.
[21] B. Saville, A scheme for the colorimetric determination of microgram amounts of thiols, Analyst 83 (1958) 670–672.
[22] H.R. Swift, D.L.H. Williams, Decomposition of S-nitrosothiols by mercury(II) and silver salts, J. Chem. Soc., Perkin Trans. 2 (1997) 1933–1935.
[23] J.Y. Park, Y.N. Lee, Solubility and decomposition kinetics of nitrous acid in aqueous solution, J. Phys. Chem. 92 (1988) 6294–6302.
[24] Q.K. Timerghazin, G.H. Peslherbe, A.M. English, Resonance description of *S*-nitrosothiols: insights into reactivity, Org. Lett. 9 (2007) 3049–3052.
[25] L.L. Perissinotti, D.A. Estrin, G. Leitus, F. Doctorovich, A surprisingly stable *S*-nitrosothiol complex, J. Am. Chem. Soc. 128 (2006) 2512–2513.
[26] M.M. Melzer, S. Mossin, A.J.P. Cardenas, K.D. Williams, S. Zhang, K. Meyer, T.H. Warren, A copper(II) thiolate from reductive cleavage of an *S*-nitrosothiol, Inorg. Chem. 51 (2012) 8658–8660.
[27] G.B. Richter-Addo, Binding of organic nitroso compounds to metalloporphyrins, Acc. Chem. Res. 32 (1999) 529–536.
[28] K.P. Kepp, A quantitative scale of oxophilicity and thiophilicity, Inorg. Chem. 55 (2016) 9461–9470.
[29] M. Laing, The Knight's Move in the periodic table, S. Afr. J. Sci. 87 (1991) 285–287.
[30] G. Rayner-Canham, M. Oldford, The chemical 'Knight's Move' relationship: what is its significance? Found. Chem. 9 (2007) 119–125.
[31] J.A.J. Pardoe, A.J. Downs, Development of the chemistry of indium in formal oxidation states lower than +3, Chem. Rev. 107 (2007) 2–45.
[32] J.H. Green, R. Kumar, N. Seudeal, D.G. Tuck, Direct electrochemical synthesis of alkane- and arenethiolato derivatives of indium and thallium, Inorg. Chem. 28 (1989) 123–127.