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Nitric Oxide Reactivity of [2Fe-2S] Clusters Leading to H_2S Generation

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Supporting Information

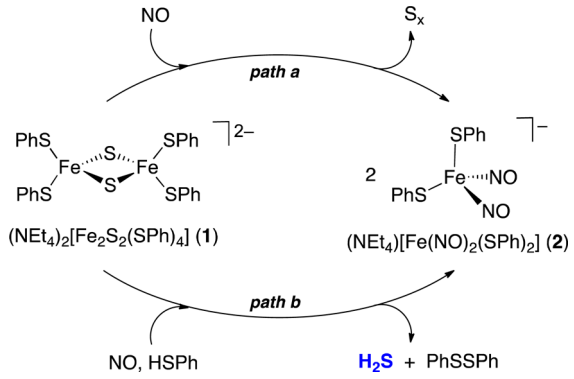
ABSTRACT: The crosstalk between two biologically important signaling molecules, nitric oxide (NO) and hydrogen sulfide (H_2S), proceeds via elusive mechanism(s). Herein we report the formation of H_2S by the action of NO on synthetic [2Fe-2S] clusters when the reaction environment is capable of providing a formal $H^•$ (e^-/H^+). Nitrosylation of $(NEt_4)_2[Fe_2S_2(SPh)_4]$ (**1**) in the presence of PhSH or $^tBu_3PhOH$ results in the formation of $(NEt_4)[Fe(NO)_2(SPh)_2]$ (**2**) and H_2S with the concomitant generation of PhSSPh or $^tBu_3PhO^•$. The amount of H_2S generated is dependent on the electronic environment of the [2Fe-2S] cluster as well as the type of $H^•$ donor. Employment of clusters with electron-donating groups or $H^•$ donors from thiols leads to a larger amount of H_2S evolution. The **1**/NO reaction in the presence of PhSH exhibits biphasic decay kinetics with no deuterium kinetic isotope effect upon PhSD substitution. However, the rates of decay increase significantly with the use of 4-MeO-PhSH or 4-Me-PhSH in place of PhSH. These results provide the first chemical evidence to suggest that [Fe-S] clusters are likely to be a site for the crosstalk between NO and H_2S in biology.

Hydrogen sulfide (H_2S)[1] has been increasingly recognized as an important signaling molecule in cardiovascular, immune, and neurological functions, which in many aspects is similar to nitric oxide (NO),[2] another well-known signaling molecule. Studies have revealed a number of biological mechanisms for the crosstalk between NO and H_2S that may explain some of the overlapping functions.[3] For example, NO and H_2S are mutually dependent on each other's presence in order to exert their angiogenic and vasorelaxant effects via converging their actions at the second messenger cGMP; NO generates cGMP by activating soluble guanylyl cyclase, whereas H_2S delays the degradation of cGMP by inhibiting phosphodiesterase-5.[4] The manner by which NO and H_2S communicate with each other, however, remains largely elusive. Efforts to gain chemical insight into this crosstalk have been made, which includes studies of the reactions of H_2S with nitroprusside,[5] S-nitrosothiols,[6] and peroxynitrite ($ONOO^-$).[7]

Inspired by the active discussions on the crosstalk between NO and H_2S, our group has begun studying the influence of the reaction environment on the formation of H_2S from [Fe-S] clusters following nitrosylation,[8] because iron−sulfur proteins are one of the main reaction sites for NO.[9] Upon nitrosylation, most [Fe-S] clusters are degraded, forming iron−nitrosyl species. While different types of iron−nitrosyls such as monomeric dinitrosyl iron complexes (DNICs)[10] and Roussin's red esters[11] have been identified as biologically relevant reaction products, the fate of the bridging sulfides (S^{2-}) during cluster modification is less clear. There are only two systems, the [4Fe-4S]-containing Wbl and FNR regulatory proteins, for which the final S-containing reaction products have been identified as sulfane (S^0) and sulfide (S^{2-}).[12] Reported here are synthetic modeling studies that suggest H_2S is a likely reaction product generated from nitrosylation of prototypical [2Fe-2S] clusters in the cellular environment.

It has long been known that synthetic [2Fe-2S] clusters react with NO to yield $\{Fe(NO)_2\}^9$ dinitrosyl iron complexes and elemental sulfur.[13,14] As previously reported,[14b,c] we too observe that gaseous NO or a chemical NO donor, Ph_3CSNO, degrades the diferric cluster $(NEt_4)_2[Fe_2S_2(SPh)_4]$ (**1**), into the $\{Fe(NO)_2\}^9$ DNIC $(NEt_4)[Fe(NO)_2(SPh)_2]$ (**2**) (path a, Scheme 1). During the conversion, the bridging sulfides of **1** provide the reducing equivalents to the $\{Fe(NO)_2\}$ unit and are released as elemental sulfur (S_x) at the end of the reaction. The amount of elemental sulfur generated can be quantified by GC-MS following conversion to its triphenylphosphine adduct, S=PPh_3.[15]

Scheme 1



We report here that the NO reactivity of $(NEt_4)_2[Fe_2S_2(SPh)_4]$ (**1**) in the presence of thiol significantly changes the fate of the bridging sulfides. When the reaction of NO(g) and **1** was carried out in the presence of benzenethiol (10 equiv), the same DNIC, $(NEt_4)[Fe(NO)_2(SPh)_2]$ (**2**), was produced as the reaction product of **1**/NO. However, only small amounts (6−7%) of elemental sulfur were found from the reaction in the presence of PhSH. Complementary to this, we observed that an additional sulfur-containing product, H_2S, was

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generated. The amount of H_2S was determined by employing a turn-on fluorescence sensor, Sulfidefluor-1 (SF1),[16] which is known to be selective for H_2S over other reactive sulfur, oxygen, and nitrogen species. The headspace gas of the reaction flask containing **1**/NO in the presence and absence of PhSH was transferred to another flask possessing an acetonitrile solution of SF1, whose fluorescence spectrum was subsequently analyzed (Figure 1). Quantitative analysis in the use of a

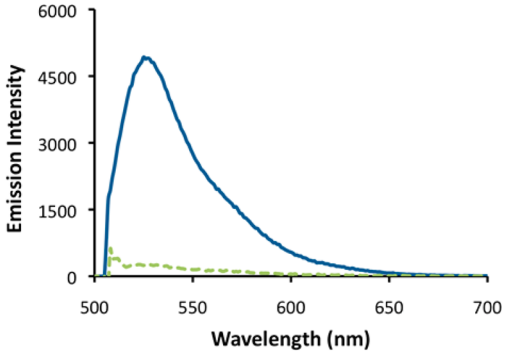

Figure 1. Fluorescence spectra of a solution of Sulfidefluor-1 upon addition of the headspace gas from the reaction of $(NEt_4)_2[Fe_2S_2(SPh)_4]$ (**1**) and NO in the absence (green dashed line) and the presence (blue solid line) of PhSH.

calibration curve created for a range of H_2S concentrations[15] revealed that ca. 80% of the bridging sulfides in **1** were released as H_2S in the presence of benzenethiol, whereas no such product was produced in the absence of externally added benzenethiol (Scheme 1, Figure 1). Additionally, we observed that the reaction of **1**/NO in the presence of PhSH produced nearly equimolar amounts of diphenyl disulfide and H_2S (i.e., 1:1 ratio of H_2S to PhSSPh).[15] This suggests that externally added benzenethiol acts as a formal $H^\bullet$ (e^-/H^+) donor to generate **2** and H_2S. In order to determine the generality of this thiol effect on H_2S production, we investigated the reactions of **1** and NO in the presence of other thiols such as EtSH and ^{t}BuSH.[17] In all cases, the reaction produced **2** and H_2S, where the amounts of H_2S generated were essentially identical with that in the reaction of **1**/NO with PhSH.[15]

In light of the H_2S production from nitrosylation of $(NEt_4)_2[Fe_2S_2(SPh)_4]$ (**1**) in the presence of thiol, we next studied the reaction of **1** and NO(g) in the presence of 2,4,6-tri-*tert*-butylphenol (^{t}Bu$_3$PhOH), another well-established $H^\bullet$ (e^-/H^+) donor with bond dissociation free energy comparable to that of PhSH (80.6 vs 76.9 kcal/mol in DMSO).[18] Similar to the reaction with thiol, nitrosylation of **1** in the presence of excess (10 equiv) ^{t}Bu$_3$PhOH led to a conversion of **1** to $(NEt_4)[Fe(NO)_2(SPh)_2]$ (**2**), during which H_2S (55%) and elemental sulfur (6%) were produced.[19] In order to confirm that ^{t}Bu$_3$PhOH provides $H^\bullet$ for the generation of H_2S and **2**, EPR spectroscopy was carried out on the reaction mixtures at room temperature (Figure 2). In the absence of ^{t}Bu$_3$PhOH, the in situ generated products from **1** and Ph_3CSNO (4 equiv) display a five-line EPR signal at g_{av} = 2.029 and $A_{N(NO)}$ = 2.4 G, as expected for the S = 1/2 system of an $\{Fe(NO)_2\}^9$ DNIC (Figure 2B).[14c,20] The EPR spectrum of the reaction products of **1** and Ph_3CSNO in the presence of ^{t}Bu$_3$PhOH (10 equiv), however, displays an additional radical signal at g = 2.004, indicating the formation of the radical ^{t}Bu$_3$PhO$^\bullet$ (Figure 2C),[21] which supports the role of ^{t}Bu$_3$PhOH as a $H^\bullet$ (e^-/H^+) donor.[22]

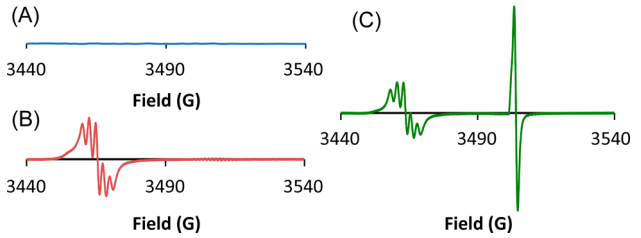

Figure 2. X-band EPR spectra obtained from the reaction of (A) **1** and ^{t}Bu$_3$PhOH, (B) **1** and Ph_3CSNO, and (C) **1** and Ph_3CSNO in the presence of ^{t}Bu$_3$PhOH in MeCN at 298 K.

The varying amounts of H_2S generated from **1** and NO by two different $H^\bullet$ (e^-/H^+) donors led us to study other factors that would play a role in H_2S generation. A series of [2Fe-2S] clusters with para-substituted benzenethiolate, $(NEt_4)_2[Fe_2S_2(SPh\text{-}4\text{-}R)_4]$, has been prepared, where R = Cl (**3**), Me (**4**), OMe (**5**). The synthesis of these clusters[14c,23] and the corresponding DNICs,[14b,c] $(NEt_4)[Fe(NO)_2(SPh\text{-}4\text{-}R)_2]$ (**6**–**8**), are known except for the methoxy analogues. Compound **5** was synthesized via a ligand exchange reaction of $(NEt_4)_2[Fe_2S_2(indolate)_4]$ with 4-methoxythiophenol in a manner similar to the synthesis of **1** and **4** reported by Meyer.[23b] The X-ray crystal structure of **5** (Figure S1, Supporting Information) reveals the bond metrics for the Fe_2S_2 rhomb of **5** to be almost identical with those reported for **1**, **3**, and **4**.[15,24] However, small changes in the $E_{1/2}$ value for $[2Fe\text{-}2S]^{2+/+}$ were observed in the series (Table 1), indicating

Table 1. Ligand Electronic Effect on H_2S Formation from NO/$[Fe_2S_2(SPh\text{-}4\text{-}R)_4]^{2-}$ in the Presence of ^{t}Bu$_3$PhOH

4-substituent (R)	amt of H_2S, %	$E_{1/2}$[a,b]
Cl (**3**)	24 ± 4	−1.36
H (**1**)	55 ± 7	−1.45
Me (**4**)	68 ± 5	−1.49
MeO (**5**)	87 ± 7	−1.50

[a]Potentials are in V vs $Cp_2Fe^{+/0}$ in MeCN at 25 °C. [b]Potentials for **1**, **3**, and **4** in DMF are known.[24a]

that the ligands affect the electronic structure of the [2Fe-2S] center. All of the [2Fe-2S] clusters with para-substituted benzenethiolate react with NO(g) or Ph_3CSNO to yield DNICs (**6**–**8**) in the absence or the presence of ^{t}Bu$_3$PhOH, but the amount of H_2S generated from the reaction in the presence of ^{t}Bu$_3$PhOH varies depending on the substituents of the cluster. Clusters having more negative reduction potentials with electron-donating groups produce larger amounts of H_2S (**3** < **1** < **4**) (Table 1), indicating that [2Fe-2S] centers in an electron-rich environment favor H_2S generation.

One of the difficulties in synthetic modeling studies of NO reactivity with [2Fe-2S] clusters lies in the concentration-dependent reactivity. As previously reported by Lippard and co-workers in detail,[14c] a DNIC and S_x are generated from nitrosylation of $(NEt_4)_2[Fe_2S_2(SPh)_4]$ (**1**) only in a concentrated solution. In contrast, dilute reaction conditions (e.g., 50 μM) generate a completely different iron product known as Roussin's black salt (RBS), $[Fe_4S_3(NO)_7]^-$, even though RBS is hardly observed biologically.[25] This reactivity pattern disappears when excess thiol is present in the reaction medium, where the bridging sulfides can be released as H_2S. Even at a concentration of 50 μM of **1**, we observe that nitrosylation of

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$(NEt_4)_2[Fe_2S_2(SPh)_4]$ (**1**) leads to the formation of the DNIC $(NEt_4)[Fe(NO)_2(SPh)_2]$ (**2**) when a large excess of PhSH (15 mM) is provided in the reaction medium.[15]

Our efforts to detect a reaction intermediate were in vain. Upon nitrosylation in the presence of 100 equiv of PhSH, we only observed a steady transformation of $(NEt_4)_2[Fe_2S_2(SPh)_4]$ (**1**) to $(NEt_4)[Fe(NO)_2(SPh)_2]$ (**2**) even at low temperatures (Figure 3A). The decay in absorbance at 480 nm from **1** is

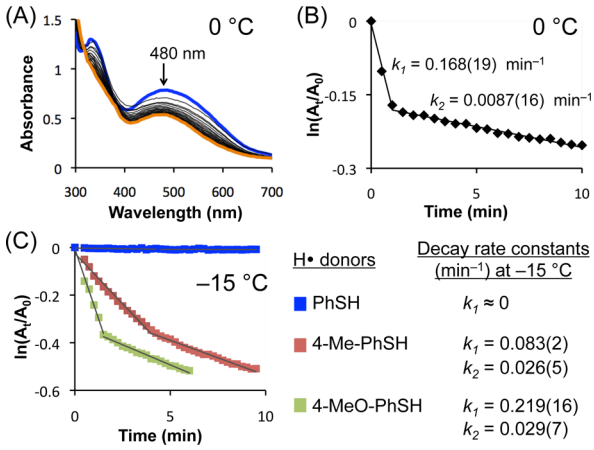
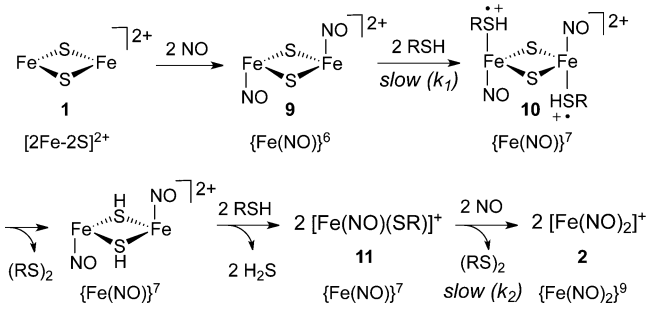


Figure 3. (A) UV−vis spectral changes for the conversion of **1** (blue) to **2** (orange) upon addition of Ph_3CSNO (6 equiv) in the presence of PhSH (100 equiv) at 0 °C over 70 min. (B) The natural log of A_{480} plotted against time at 0 °C, where A_0 and A_t = absorbance at 480 nm at t = 0 and t min, respectively. (C) Comparison of decay kinetic traces for the conversion of **1** to **2** upon addition of Ph_3CSNO (6 equiv) in the presence of 100 equiv of PhSH (blue), 4-Me-PhSH (red), and 4-MeO-PhSH (green) at −15 °C. The initial concentration of **1** is 3.6×10^{-4} M in acetonitrile for all.

found to be biphasic, where the first phase is faster than the second, which can be fit to two consecutive first-order decays to give k_1 = 0.168(19) min^{-1} and k_2 = 0.0087(16) min^{-1} at 0 °C (Figure 3B). No deuterium kinetic isotope effect was observed when PhSH was replaced by PhSD, indicating proton transfer is not involved in the rate-limiting step. However, the presence of water, which can potentially compete with PhSH or NO in binding to Fe,[26] influences the decay rate. When small amounts of H_2O (700 equiv per **1**) were added to the reaction medium, the first decay process was slowed down by ∼1.5-fold at 0 °C (not shown).[15] The rates of decay were also found to be sensitive to the electronic nature of H• donors. The employment of para-substituted benzenethiol with electron-donating MeO and Me groups led to a notably faster decay, although neither the starting cluster **1** nor the final product **2** reacts with these substituted benzenethiols. At −15 °C, at which the reaction of **1**/NO in the presence of PhSH barely begins to proceed, the same reaction in the presence of 4-MeO-PhSH and 4-Me-PhSH were completed in less than 10 min (Figure 3C).

Our current working model for a plausible reaction pathway is shown in Scheme 2, in which the very last step, the conversion of **11** to **2**, is adopted from a known reaction.[14c] The presence of H• donors such as thiols and phenols in the environment is crucial in generating H_2S. However, the H• donors tested here have no reactivity with the starting [2Fe-2S] clusters. This suggests that the initial reaction between NO and the [2Fe-2S] clusters would likely produce an oxidizing iron−nitrosyl intermediate such as **9** (Scheme 2) that is capable of

Scheme 2. Current Working Model[a]



[a]Each Fe has two additional thiolate ligands (not shown).

abstracting a formal H• (e^-/H^+) from benzenethiol.[27] The increased decay rates upon employing benzenethiol with electron-donating substituents led us to conjecture that the reaction mechanism must have multiple electron transfer steps and reduction of iron nitrosyl moieties by thiol or thiolate, such as the conversions of **9** to **10** and **11** to **2**, is likely important in determining the overall reaction rates.

The present studies demonstrate that the degradation of prototypical [2Fe-2S] clusters by NO in the presence of H• (e^-/H^+) produces H_2S. Proton-coupled electron transfer (PCET) by cellular H• donors such as cysteine and tyrosine is prevalent in biology. The importance of PCET reactivity of iron−sulfur clusters has been widely appreciated in systems such as CO-ligated [Fe-S] hydrogeneases[28] and the Reiske proteins.[29] Our results here strongly suggest that the NO reactivity of prototypical cysteinate-bound [Fe-S] clusters is likely coupled to PCET chemistry, in which local protein residues or the millimolar concentrations of intracellular glutathione[30] likely play a role in [Fe-S] degradation by NO leading to the formation of H_2S. Therefore, it is conceivable that iron−sulfur clusters might be one of the intersecting sites that facilitate crosstalk between NO and H_2S.

■ ASSOCIATED CONTENT

Supporting Information

Text, figures, tables, and a CIF files giving experimental details and characterization data for all compounds prepared and crystallographic data for **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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