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Acid-Dependent Degradation of a [2Fe−2S] Cluster by Nitric Oxide

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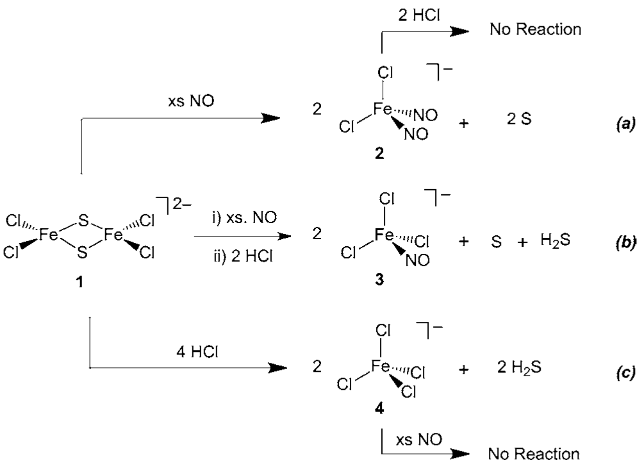
Ⓢ *Supporting Information*

ABSTRACT: New types of degradation products of iron−sulfur clusters by nitric oxide (NO) have been identified in the acidic environment. In the absence of acid, NO reacts with $(Et_4N)_2[Fe_2S_2Cl_4]$ (**1**) to form a $\{Fe(NO)_2\}^9$ dinitrosyliron complex, $(Et_4N)[Fe(NO)_2Cl_2]$ (**2**), wherein the bridging sulfides are oxidized to elemental sulfur by four electrons ($2S^{2-} \rightarrow 2S^0 + 4e^-$). In contrast, the successive additions of NO and HCl to **1** result in the formation of a $\{Fe(NO)\}^7$ mononitrosyliron complex, $(Et_4N)[Fe(NO)Cl_3]$ (**3**), along with elemental sulfur and hydrogen sulfide (H_2S), which are the two-electron-oxidized products of the bridging sulfides ($2S^{2-} + 2H^+ \rightarrow H_2S + S^0 + 2e^-$). The results demonstrate that the acidic environment plays a significant role in controlling the chemistry of an iron−sulfur cluster with NO and imply how two important gaseous molecules, NO and H_2S, can be interconnected through iron−sulfur clusters.

Nitric oxide (NO) is a ubiquitous signaling molecule[1] that plays an important role in many physiological functions such as blood-pressure regulation,[2] neurotransmission,[3] and immune response.[4] In the past decade, iron−sulfur proteins have been identified as one of the main targets of NO.[5] Although the global degradation of [Fe−S] clusters by NO is an indication of NO cytotoxicity,[6] this type of modification is also used as a genetic switch in signal transduction.[7] The most commonly observed degradation product from the reaction between [Fe−S] clusters and NO is the thiolate-bound dinitrosyliron complex (DNIC) in the form of $[Fe(NO)_2(SR)_2]^-$.[8] However, other types of reaction products, such as an electron paramagnetic resonance silent thiolate-bridged $[Fe_2(\mu\text{-}SR)_2(NO)_4]$ species, have also been observed.[7b,9] We are interested in understanding how the environment influences the NO reactivity of [Fe−S] clusters because the bonding nature of [Fe−S] clusters is known to be sensitive to the local environment such as solvent accessibility and hydrogen bonding;[10] this led us to postulate that the acidity may play a role in the degradation of [Fe−S] clusters by NO. We report here the unprecedented NO reactivity of a [2Fe−2S] cluster in the presence of acid, which leads to the generation of two new types of cluster degradation products, a mononitrosyliron complex (MNIC) and hydrogen sulfide (H_2S).

We chose a known[11] cluster, $(Et_4N)_2[Fe_2S_2Cl_4]^{2-}$ (**1**), as a model system for this study because the distinctive UV−vis features[11c] of **1** can serve as an effective indicator to monitor the cluster degradation, the redox-innocent Cl^- ligand simplifies the system, and various iron nitrosyl compounds with Cl^- ligands, possible degradation products, are also known as stable compounds.[12] Compound **1** was synthesized according to the literature procedure.[11b] The synthesis of $(Et_4N)[Fe(NO)_2Cl_2]$ (**2**) as a synthon for various DNICs has been previously explored by Tonzetich et al.[12a] As reported,[12a] injecting either a stoichiometric or an excess (ca. 10 equiv) amount of NO into an acetonitrile solution of **1** at room temperature leads to the formation of **2**, which can be easily identified by its characteristic ν_{NO} stretching frequencies at 1768 and 1697 cm^{-1} (Scheme 1a and Figure 1). During the

Scheme 1



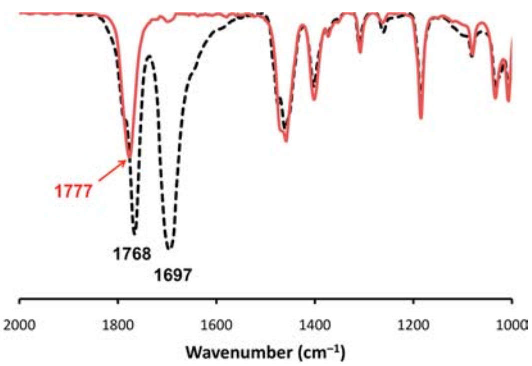


Figure 1. IR spectra (in KBr) of the crude product from the reaction of **1** with NO to form **2** (black dashed) and the reaction of **1** with NO and 2 equiv of HCl to form **3** (red solid).

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reaction, the bridging sulfides in **1** are reductively eliminated as elemental sulfur, which was analyzed from its triphenylphosphine adduct by gas chromatography−mass spectrometry (GC−MS), a common method used for sulfur detection.[13] From reaction (a) (Scheme 1), compound **2** was isolated in 96% yield, and 1.2 equiv of $S{=}PPh_3$ (per cluster) was detected by GC−MS analysis.[14] The observed NO reactivity of **1** in the *absence* of acid is exactly analogous to those for the known [2Fe−2S] clusters with thiolate ligands, in which $\{Fe(NO)_2\}^9$ DNICs are generated, with the necessary reducing equivalents provided from the bridging sulfides ($2S^{2-} \rightarrow 2S^0 + 4e^-$).[12a,13b,15,16]

The presence of acid drastically alters the reaction products. We chose HCl as the acid source because its conjugate base, Cl^-, is identical with the ligand of the starting cluster, **1**. This simplifies the system by avoiding possible complications that may arise from potential ligand substitution. When NO is introduced to **1** with the successive addition of 2 equiv of HCl at room temperature, a MNIC, $(Et_4N)[Fe(NO)Cl_3]$ (**3**), is generated as the major iron compound with a ν_{NO} stretching frequency at 1777 cm^{-1} (Figure 1) and a UV absorption band at 270 nm. We have independently synthesized and characterized compound **3** for unambiguous product identification,[17] although $[Fe(NO)Cl_3]^-$ complexes formed with other types of cations have been previously known.[12b−d] Compound **3** was isolated in 92% yield from reaction (b). There was no hint of the generation of **2** in the reaction products even when a large excess of NO was used.[18]

The generation of a $\{Fe(NO)\}^7$ MNIC, **3**, from reaction (b) (Scheme 1), as opposed to a $\{Fe(NO)_2\}^9$ DNIC, led us to investigate the fate of the bridging sulfides in **1**. The amount of elemental sulfur was again quantified by GC−MS analysis of $S{=}PPh_3$. Compared to reaction (a), half the amount of $S{=}PPh_3$ (i.e., 0.6 equiv per cluster) was detected from reaction (b). Complementary to this result, an additional sulfur-containing product, H_2S, was observed during the reaction. H_2S evolution was first qualitatively noticed from the headspace gas of the reaction flask with a commercial dosimeter detection tube (GASTEC, Japan) or a lead acetate paper strip (Sigma).[17] In order to quantify the amount of H_2S generated, we have synthesized a recently reported turn-on fluorescence sensor, Sulfidefluor-1 (SF1), which is known to be selective for H_2S over reactive sulfur, oxygen, and nitrogen species.[19] The headspace gas of the **1**/NO/HCl reaction [reaction (b)] was transferred to an acetonitrile solution containing SF1, whose fluorescence spectrum was subsequently analyzed. While the **1**/NO reaction [reaction (a)] did not induce any fluorescence, reaction (b) led to a significant fluorescence enhancement at 525 nm (Figure 2), indicative of the presence of H_2S. Further quantitative analysis in the use of a calibration curve showed that 0.76 equiv of H_2S (per cluster) was evolved from reaction (b).[17] This result combined with GC−MS analysis of $S{=}PPh_3$ suggests that the bridging sulfides in **1** are transformed to elemental sulfur and H_2S in a 1:1 ratio in reaction (b).

In the absence of NO, **1** reacts with 4 equiv of HCl to form $(Et_4N)[Fe^{III}Cl_4]^-$ (**4**) and H_2S (Scheme 1c and Figure 2). About 2 equiv of H_2S (per cluster) was detected from reaction (c), with no indication of the elemental sulfur formation. Complex **4** was isolated in 77% yield and was identified from its known[20] UV−vis characteristics and by electrospray ionization mass spectrometry.

The chemistry observed is clearly dependent on the order and time in which the reactants are added. In order to control the production of **3** versus **2** or **4**, NO has to be added to the solution of **1** prior to acid in succession (Scheme 1). Reversing the order, i.e., HCl addition followed by NO addition, leads to cluster degradation to **4**. Delay in the acid addition produces **2**. Once compound **2** or **4** is formed, they are not able to transform to **3** upon HCl or NO addition, suggesting that neither **2** nor **4** can be an intermediate in the route to the formation of **3** in reaction (b). The results obtained from this study suggest that the initial interaction between NO and **1** likely generates an iron nitrosyl intermediate associated with a $2e^-$ oxidation of the bridging sulfides (i.e., electronically equivalent to $2S^{2-} \rightarrow S^{2-} + S^0 + 2e^-$) prior to DNIC formation. The presence of HCl may then alter the reaction path for the intermediate, leading to the formation of **3**, elemental sulfur, and H_2S.[21]

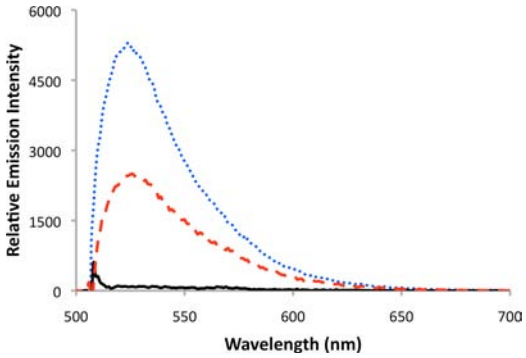

Figure 2. Fluorescence spectra of SF1 upon the addition of the headspace gas of the reaction of **1** and NO [reaction (a): black solid], the reaction of **1**, NO, and HCl [reaction (b): red dashed], and the reaction of **1** and HCl [reaction (c): blue dotted], with the concentration of **1** = 0.035 M for all reactions.

The generation of MNICs from the interaction of NO and nonheme iron has been observed biologically[22] and chemically.[13b,23] The current study demonstrates that iron−sulfur clusters could be a source for MNIC generation in the acidic environment. This provides new insight into the reactivity of [Fe−S] clusters with NO, in which $\{Fe(NO)_2\}^9$ DNICs, in the form of either mononuclear or dinuclear species, have been recognized as the predominant form of the iron-containing reaction product.[9,12a,13b,15] Furthermore, the current study shows that H_2S can be generated from the interaction between NO and an iron−sulfur cluster for the first time. Both NO and H_2S are important gasotransmitters[1,24] whose signaling pathways are known to interact on various levels.[24] Chemical investigations on the cross-talk between NO and H_2S have just begun to appear in the literature.[25] We expect that the reactivity of NO with biologically relevant [Fe−S] clusters in the acidic environment would be more complicated than the chemistry observed with **1**, in which the presence of redox-active cysteinate (thiolate) could play a role and alter the reaction path. Nonetheless, the present work provides a foundation for exploring more elaborate systems in order to probe the possible role(s) of iron−sulfur clusters in mediating the cross-talk between NO and H_2S.

In summary, this study identifies the new types of [Fe−S] cluster degradation products by NO in the acidic environment. In the presence of acid, a four-coordinate MNIC species is generated via [2Fe−2S] cluster degradation by NO, during which one of the bridging sulfides is released as H_2S. Further studies will expand our investigation to more biologically relevant synthetic analogues and study their reactivity with NO in the presence of acid.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details for the synthesis, spectroscopy, reaction product characterization, and analysis of **1**−**4**, elemental sulfur, and H_2S. 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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