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Transformation of a Mononitrosyl Iron Complex to a [2Fe-2S] Cluster by a Cysteine Analogue

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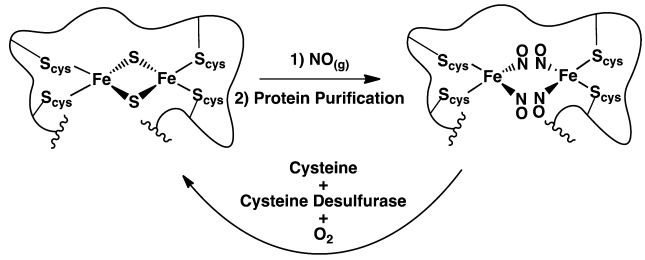
Supporting Information

ABSTRACT: Reversible modification of iron-sulfur clusters by nitric oxide acts as a genetic switch in a group of regulatory proteins. While the conversion of [Fe-S] clusters to iron-nitrosyls has been widely studied in the past, little is known about the reverse process, the repair of [Fe-S] clusters. Reported here is a system in which a mononitrosyl iron complex (MNIC), (PPN)[Fe(S^tBu)$_3$(NO)] (**1**), is converted to a [2Fe-2S] cluster, (PPN)$_2$[Fe_2S_2($SCH_2CH_2C(O)OMe$)$_4$] (**2**). This conversion requires only the addition of a cysteine analogue, 3-mercaptomethylpropionate (MMP), at room temperature without the need for any other reagents. The identity of **2** was confirmed spectroscopically, chemically, crystallographically, and analytically. Mass spectrometry and ^{34}S labeling studies support that the bridging sulfides in **2** derive from the added MMP, the cysteine analogue. The NO lost during the conversion of **1** to **2** is trapped in a dinitrosyl iron side product, (PPN)[Fe($SCH_2CH_2C(O)OMe$)$_2$(NO)$_2$] (**4**). The present system implies that MNICs are likely intermediates in the repair of NO-damaged [2Fe-2S] clusters and that cysteine is a viable molecule responsible for the destabilization of MINCs and the formation of [2Fe-2S] clusters.

Nitric oxide (NO) plays a well-established role in physiological systems by acting as a regulator of vasodilation, neurotransmission, platelet aggregation, and the cell-mediated immune response.[1] NO is capable of interacting with a variety of chemical groups found in biological systems, although the most prominent are thiols,[2] to form nitrosothiols, and metal centers,[2b,3] to form metal nitrosyls. Iron-sulfur clusters are protein cofactors that are known to interact with NO leading to either elimination[4] or initiation[5] of biological activity. Some [Fe-S]-containing proteins initiate gene regulation upon reaction with NO.[6] One of the best-studied examples is the *Escherichia coli* protein SoxR, in which NO modification of the [2Fe-2S] cluster produces SoxR-bound dinitrosyl iron complexes (SoxR-DNICs) and upregulates the expression of more than 60 defense genes.[5b,7] When NO is removed, the SoxR-DNICs are efficiently converted back to the [2Fe-2S] protein by a mechanism that remains unknown *in vivo*.[5b]

A DNIC to [2Fe-2S] cluster repair process is crucial if the interconversion between these two species constitutes a biologically relevant signaling mechanism. Additionally, the repair chemistry has significance in understanding how cells might cope with global NO stress that leads to a significant occurrence of [Fe-S] cluster degradation to DNICs beyond NO-sensing regulatory proteins. Ding et al. have shed light on this process using a [2Fe-2S] cluster containing ferredoxin (Fdx). Protein-bound DNIC formed by treating Fdx with NO can be converted back to the holo-form following incubation with cysteine desulfurase, cysteine, and dioxygen (Scheme 1).[8]

Scheme 1. *In Vitro* Transformation of [2Fe-2S] Cluster and DNIC in Ferredoxin



Cysteine desulfurase is an enzyme that catalyzes the conversion of cysteine to alanine and a protein-bound persulfide (RS_{cys}-SH).[9] The resulting persulfide sulfur is then used as a source of sulfur in a number of protein cofactors including [Fe-S] clusters.

The details of the chemistry governing the cysteine desulfurase-mediated DNIC repair remain unknown, although synthetic model systems have been constructed to provide information about the DNIC to [2Fe-2S] cluster transformation.[10] Model systems reported by Lippard[10b] and Liaw[10c] describe the photolytic conversion of iron nitrosyl to [2Fe-2S] cluster in the presence of elemental sulfur. Another system investigated by the Liaw group exhibits the ability to

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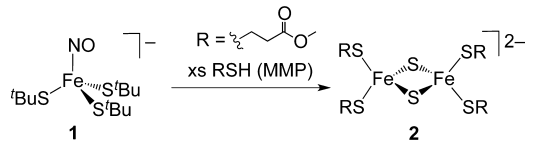
convert from DNIC to [2Fe-2S] cluster on sequential addition of tritylthiol and an NO-sequestering iron tetrathiolate.[10a] While providing valuable insight, these systems have utilized reagents and reaction conditions that are significantly different from the repair observed in proteins. We report here the unprecedented transformation of MNIC to [2Fe-2S] cluster requiring only the addition of a cysteine analogue (Scheme 2) and bring to light the previously unexplored potential of MNICs to act as intermediates in the repair of [2Fe-2S] clusters from DNICs.

Scheme 2. Transformation of MNIC to [2Fe-2S] Cluster



Incubation of (PPN)[Fe(S^{t}Bu)$_3$(NO)] (**1**),[11] an MNIC bearing *tert*-butylthiolate ligands where PPN = μ-nitrido-bis(triphenylphosphorus), with excess methy 3-mercaptopropionate (MMP) resulted in two types of Fe-containing reaction products, one of which is a [2Fe-2S] cluster bearing MMP thiolate ligands, (PPN)$_2$[Fe$_2$S$_2$(SCH$_2$CH$_2$C(O)OMe)$_4$] (**2**), Scheme 2. The careful fractional purification of the reaction mixture enabled us to identify every reaction product (*vide infra*). Specifically, a solution of **1** was treated with excess (20 equiv) MMP at room temperature for 6 h in the dark. After removing solvent *in vacuo*, the reaction mixture was washed with diethyl ether and then THF. The solid that was insoluble in the THF wash was recrystallized from acetonitrile/diethyl ether to give **2** as a dark-brown microcrystalline solid (45%). Although the microcrystalline solid appears stable for months if stored at −38 °C, the cluster rapidly decomposes in solution, even at low temperatures. The presence of excess MMP in solution, however, is able to stabilize the cluster significantly.[12]

Solutions of **2** exhibit UV−vis absorption maxima at 330, 425, and 450 nm in acetonitrile, which are highly reminiscent of those of ethylthiolate- and protein-bound [2Fe-2S] clusters.[13] The IR spectrum of the solid material has an intense sharp band at 1727 cm^{-1}, which arises from the carbonyl unit in MMP. Cyclic voltammetry experiments using a glassy carbon electrode indicate that an electrochemically quasi-reversible [2Fe-2S]$^{2+/1+}$ event occurs at −870 mV vs Ag/AgNO$_3$ in acetonitrile. Finally, diffraction quality crystals of the compound were grown from a concentrated acetonitrile solution of **2** in the presence of excess MMP cooled to −38 °C and X-ray diffraction analysis permitted solution of the crystal structure (Figure 1). The bond metrics of the Fe$_2$S$_2$ rhomb are comparable to those reported for analogous structures.[14]

Despite its instability, **2** can undergo expected substitution of the peripheral MMP thiolates for phenylthiolate ions to quantitatively produce (PPN)$_2$[Fe$_2$S$_2$(SPh)$_4$] (**3**), a previously reported and well-characterized stable [2Fe-2S] cluster.[15] This reactivity not only serves to further confirm the formation of **2** but also provides a means of transferring the Fe$_2$S$_2$ rhomb into a more stable molecular framework on which to carry out subsequent characterization. The phenyl-substituted cluster can be characterized by electrospray ionization mass spectrometry (ESI-MS) and we capitalized on this feature to probe the origin of the bridging sulfide. Given the starting reagents, the bridging sulfide can only come from either the *tert*-butylthiolate groups

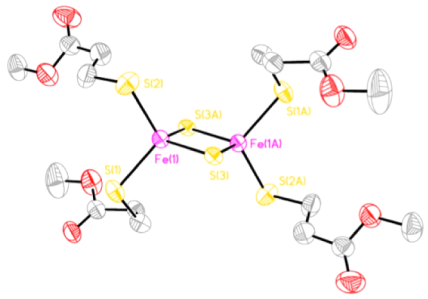


Figure 1. ORTEP diagram of the anion of (PPN)$_2$[Fe$_2$S$_2$(SCH$_2$CH$_2$C(O)OMe)$_4$] (**2**) with 50% thermal ellipsoids. Hydrogen atoms are omitted for clarity.

of **1** initially bound to the Fe or from the added MMP. In order to distinguish between two possibilities, the starting MNIC, (PPN)[Fe(S^{t}Bu)$_3$(NO)] (**1**), was prepared using ^{34}S-labeled *tert*-butylthiolate and subsequently used in the preparation of **2**. This cluster was then converted to **3** and analyzed by ESI-MS. The data clearly indicate that the bridging sulfides in **3** are not enriched in ^{34}S (Figure S6). The possibility that the sulfur atoms of the bridging sulfides exchange with those of the phenylthiol used to derivatize the cluster was discounted by carrying out the derivatization with Ph^{34}SH in which a mass shift of 8 was observed indicating the formation of (PPN)$_2$[Fe$_2$(μ-S)$_2$(^{34}SPh)$_4$] (Figure S7). Moreover, we observed that the *tert*-butylthiolate ligands on **1** are released as *tert*-butylthiol by ^{1}H NMR spectroscopy (Figure S5). The only other source of the sulfide in the Fe$_2$S$_2$ rhomb is the excess MMP present in solution.

Analysis of the organic material washed from the product of the reaction of (PPN)[Fe(S^{t}Bu)$_3$(NO)] (**1**) with MMP suggests the manner by which the sulfur atom is removed from the latter. Gas chromatography mass spectrometry (GC-MS) measurements of the diethyl ether washings indicate that the thioether S(CH$_2$CH$_2$C(O)OMe)$_2$ is formed in the reaction. GC-MS quantification of the amount of thioether formed over the course of the reaction confirms that it is produced in a 2:1 stoichiometry with the product cluster. Specifically, on average 93% of the expected thioether is produced.

The fate of the NO that is lost on transformation from (PPN)[Fe(S^{t}Bu)$_3$(NO)] (**1**) to (PPN)$_2$[Fe$_2$S$_2$(SCH$_2$CH$_2$C(O)OMe)$_4$] (**2**) becomes apparent on investigation of the THF-soluble side product described above. Addition of pentane to the THF solution of this side product resulted in the deposition of an oily residue. Spectroscopic analysis of the residue is consistent with its formulation as (PPN)[Fe(SCH$_2$CH$_2$C(O)OMe)$_2$(NO)$_2$] (**4**), a DNIC bearing two MMP thiolate ligands. The EPR spectrum of the side product shows the characteristic g = 2.03 signal expected to arise for a strongly coupled S = 1/2 {Fe(NO)$_2$}^{9} DNIC.[16] ESI-MS revealed the compound to have m/z = 354.4 for [**4**-PPN]$^-$ consistent with its formulation. The IR spectrum of the isolated product shows bands at 1715 and 1670 cm^{-1}, which coincide with those of independently prepared **4**.

The only final destination of NO derived from (PPN)[Fe(S^{t}Bu)$_3$(NO)] (**1**) appears to be the DNIC, (PPN)[Fe(SCH$_2$CH$_2$C(O)OMe)$_2$(NO)$_2$] (**4**). EPR monitoring of the reaction solution shows that the g = 2.03 signal from **4** grows in over the course of the reaction (Figure 2). No other NO-containing byproduct has been observed by EPR or IR spectroscopy nor the formation of N$_2$O was detected in the

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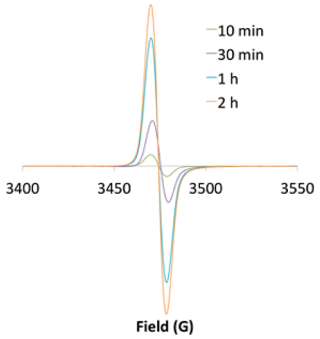
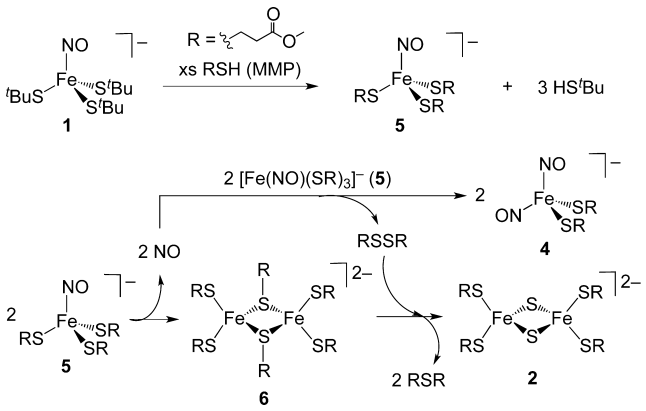


Figure 2. Room temperature X-band EPR spectral changes associated with the formation of (PPN)[$Fe(SCH_2CH_2C(O)OCH_3)_2(NO)_2$] (**4**) from the reaction of (PPN)[$Fe(S^tBu)_3(NO)$] (**1**) and MMP in acetonitrile.

headspace of the reaction mixture. Moreover, the isolated yield of **4** indicates that it comprises ~50% of the Fe present in **1**. A 2:1 stoichiometry of the DNIC and [2Fe-2S] cluster, **4** and **2**, is consistent with a scheme in which these two complexes are the only significant Fe- and NO-containing species formed during the reaction (Scheme 3).

Scheme 3. Working Model of the Transformation of MNIC into [2Fe-2S] Cluster by MMP



The experiments presented above provide insight into the events that may occur during the chemical transformation of MNIC to [2Fe-2S] cluster in the presence of MMP. A reaction model (Scheme 3) has been proposed based on evidence for the formation of [2Fe-2S] cluster, DNIC, and MMP-thioether in a 1:2:2 molar ratio. The S^{t}Bu ligands on (PPN)[$Fe(S^tBu)_3(NO)$] (**1**) are first substituted by MMP to initiate the reaction, as evidenced by release of HS^{t}Bu, forming the MMP thiolate-bound MNIC, **5**. In separate experiments, nearly identical chemistry was observed in the reaction of (PPN)[$Fe(SEt)_3(NO)$] with MMP, whereas no reaction was observed with (PPN)[$Fe(SPh)_3(NO)$] or (PPN)[$FeCl_3(NO)$] because MMP could not replace the original ligands of these MNICs (data not shown). Electronic structure calculations suggest that four-coordinate **5**, as shown in Scheme 3, is likely in equilibrium with a five-coordinate isomer in which the carbonyl moiety of the MMP ligand interacts with the Fe center (see SI). This feature is unique to MMP among the thiols tested and is likely important in the overall reactivity of this species. The electronic structure of four-coordinate **5** can be described as a high-spin Fe^{3+} center antiferromagnetically coupled to an NO^- ligand, as suggested for **1** by Lippard et al.[11] Ligation of an MMP carbonyl moiety, forming five-coordinate **5**, yields a species with an electronic structure that is better described as a high-spin Fe^{2+} center antiferromagnetically coupled to a neutral NO• ligand. Furthermore, the NO ligand is *trans* to a labilizing thiolate.[17] Both of these influences should lead to more facile NO release from the iron-center.

We presume that **5** is unstable and disproportionates into a more stable DNIC, **4**, and a thiolate-bridged diferrous compound, **6** (Scheme 3). Thiolate-bridged species analogous to **6** have been previously reported in the literature and shown to form [2Fe-2S] clusters on reaction with elemental sulfur.[18] In our proposed reaction, the diferrous **6** is converted to the diferric **2** by using one equivalent of disulfide (RSSR) with generation of two equivalents of thioether (RSR) as a byproduct. Support for the feasibility of the reaction between **6** and MMP disulfide came from our independent investigation on the reaction between $(NEt_4)_2[Fe_2(\mu\text{-}SEt)_2(SEt)_4]$,[18b] an analogue of **6**, and excess MMP disulfide. In this reaction, clean formation of **2** and the corresponding MMP thioether was observed (see SI). Alternatively, **2** might form via a different intermediate, $[Fe_2(\mu\text{-}SR)_2(SR)_2(NO)_2]^{2-}$, the Ni analogue of which is known.[20,21] We were not, however, able to synthesize the iron analogue in order to test the feasibility of this hypothesis. Further mechanistic investigations are beyond the scope of this report and will be the subject of future studies.

The present studies suggest a new potential role of MNICs as intermediate species in the repair of [2Fe-2S] clusters following nitrosylation. In 2006, Lippard first suggested that MNICs could be involved in reaction pathways leading to DNIC formation in biologically relevant sulfur-rich coordination environments.[11] Here we demonstrate the first one-pot conversion of a mononuclear iron-nitrosyl species to [2Fe-2S] cluster requiring no reagents other than a cysteine analogue. The *in vitro* transformation of protein-bound DNICs into functional [2Fe-2S] clusters is reported to require cysteine, cysteine desulfurase, and dioxygen.[8] Although MNICs were not initially implicated in this biological transformation, the results presented here suggest that they may play a role in it and that MNICs may, in general, have a greater biological importance than is typically ascribed to them. Future work is geared toward understanding other aspects of [2Fe-2S] cluster repair chemistry, including the conversion of DNIC to MNIC and the role of dioxygen.

■ ASSOCIATED CONTENT

Supporting Information

Experimental and computational details, UV−vis, FTIR, ESI-MS, and ^{1}H NMR spectra, cyclic voltammogram of **2**, calibration curve for dimethyl 3,3′-thiopropionate, CIF for **2**. 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.

■ ACKNOWLEDGMENTS

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(21) For example, 4 equiv of MMP-thiolate may react with $[Fe_2(\mu\text{-}SR)_2(SR)_2(NO)_2]^{2-}$ to generate **2**, 2 equiv of thioether, and NO^-.