

H₂S/Thiols, NO[•], and NO⁻/HNO: Interactions with Iron PorphyrinsSilvina Bieza,[†] Agostina Mazzeo,[†] Juan Pellegrino, and Fabio Doctorovich*Cite This: *ACS Omega* 2022, 7, 1602–1611

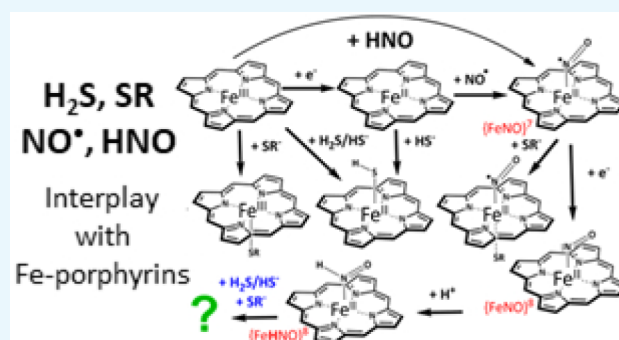
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ABSTRACT: In the past decade, gasotransmitters NO[•] and H₂S have been thoroughly studied in biological contexts, as their biosynthesis and physiological effects became known. Moreover, an additional intricate *crossstalk* reaction scheme between these compounds and related species is thought to exist as part of the cascade signaling processes in physiological conditions. In this context, heme enzymes, as modeled by iron porphyrins, play a central role in catalyzing the key interconversions involved. In this work, iron porphyrin interactions with sulfide and nitric-oxide-related species are described. The stability and reactivity of mixed ternary systems are also described, and future perspectives are discussed.



INTRODUCTION

The discovery of its biosynthesis and the assertion of its physiological effects have made nitric oxide (NO[•]) a

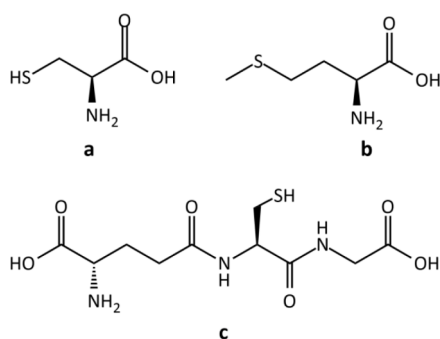


Figure 1. Most common biologically relevant sulfur species: (a) cysteine, (b) methionine, and (c) glutathione.

biologically relevant species. In mammals, this molecule acts as a mediator in the cardiovascular, nervous, and immune systems, mainly via interaction with its natural receptor, soluble guanylyl cyclase (sGC). This is a heme enzyme, displaying an iron-bound porphyrin active site just like many enzymes of the nitrogen cycle which deal with NO-related species: nitric oxide synthase (NOS, responsible for endogenous NO[•] production), fungal CytP450 nitric oxide reductase (P450nor, which converts it to N₂O), and more. Other small nitrogen species also present biologically relevant reactivity, such as nitroxyl (NO⁻ or HNO, the one-electron reduction products of NO[•]), the nitrosonium cation (NO⁺), and peroxyxynitrite (ONOO⁻). In particular, nitroxyl has recently been established to produce

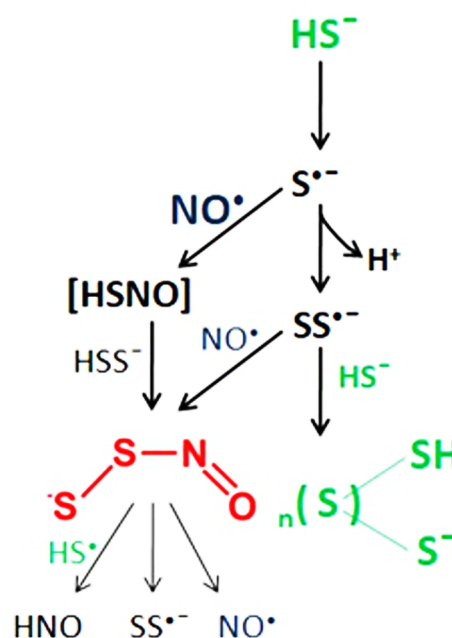


Figure 2. Cascading chemical reactions with generation of radical intermediates in the absence of oxygen (adapted with permission from ref 6).

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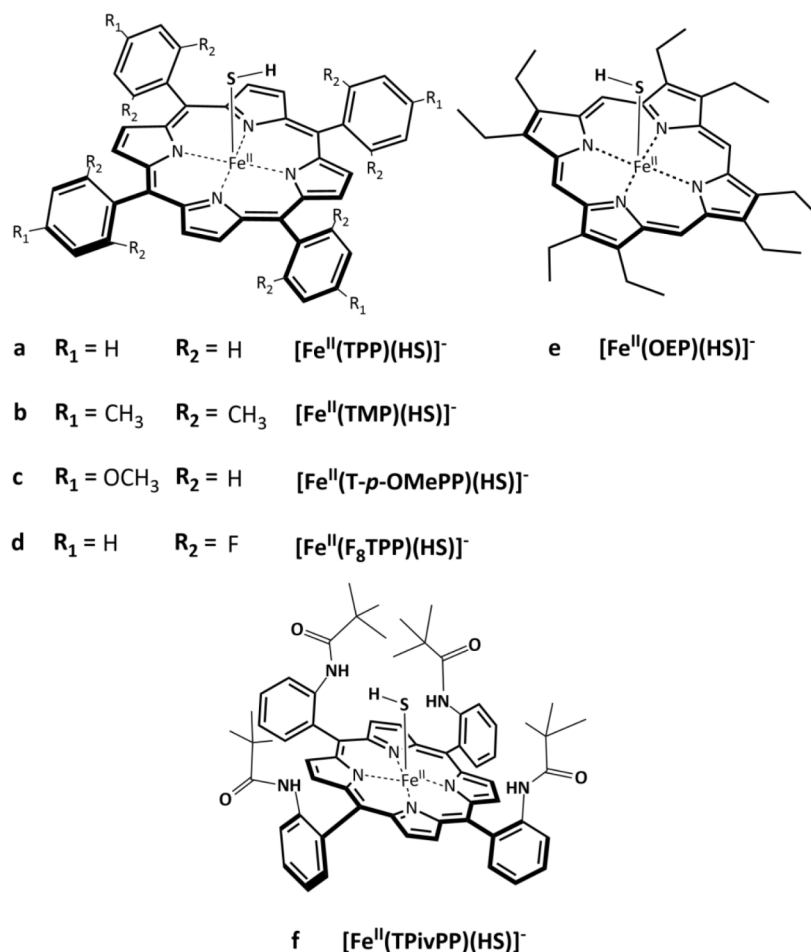


Figure 3. Pentacoordinate sulfide complexes obtained with different iron porphyrins.

its own physiological effects—both similar and distinct from those of NO^\bullet —and though it could be produced from different metabolites, the question of its biosynthesis remains unsolved.¹

Biologically relevant sulfur species, on the other hand, consist mainly of thiols, as in cysteine (Cys, Figure 1a) and methionine (Met, Figure 1b) amino acids in proteins or as the antioxidant tripeptide glutathione (GSH, Figure 1c).

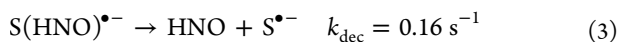
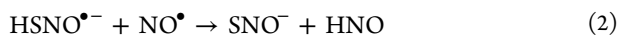
Despite the extent of studies regarding the biologically relevant reactivity of those sulfur-based ligands, across many subdisciplines from organometallics and bioinorganic chemistry to cluster science, studies on the hydrosulfide ion (HS^-) are significantly less developed. Biological roles for this ion, however, have revolved around its coordination with transition metal ions that produce a unique class of effects that differ from metal thiolates. Conventionally, dihydrogen sulfide (H_2S) was considered toxic until more than 20 years ago when Kimura suggested its possible physiological role in the nervous system. Since then, there is evidence that H_2S plays an important role in several mammalian processes, such as vascular contractility (and thus its function in blood pressure regulation) and neuronal activity, among others.² The endogenous H_2S production in mammalian tissues is a consequence of the action of at least three enzymes: cystathionine- γ -lyase (CSE), cystathionine- β -synthase (CBS), and 3-mercaptopyruvate-sulfuryltransferase (MPST). The enzymes CSE and CBS use cysteine as the main substrate.

Both NO^\bullet and H_2S are now considered major intracellular signaling molecules, part of the *gasotransmitters* family, along with carbon monoxide (CO). They are defined as endogenous, gaseous molecules whose metabolism is strictly regulated and have a defined biological role at low concentrations, although they are toxic at high concentrations. In particular, NO^\bullet and H_2S display notable chemical reactivity because of their mutual involvement in numerous cellular processes.³ After long speculation, the interaction between these two species has been demonstrated, and they were found to influence the production of each other as well as the expression and activity of the enzymes responsible for their biosynthesis.⁴ In addition, the inhibition of vasorelaxant effects of NO^\bullet in the presence of H_2S was attributable to a possible biochemical “crosstalk” between the two gases, even though other authors report that H_2S reduces such activity.⁴ As a result, interest in the possible link between H_2S and NO^\bullet to better understand their biological functions has recently dramatically increased. It has also been suggested that nitroxyl could be endogenously generated via the reaction of these two gasotransmitters.⁵ H_2S is capable of transforming endogenous NO^\bullet to HNO , being involved in blood pressure and cardiac contractility control. This is because HNO activates the HNO /transient receptor potential channel/calcitonin gene-related peptide cascade, via the formation of disulfide bonds, resulting in continuous calcium influx.

Reaction of H_2S with *S*-nitrosothiols (RSNOs) forms thionitrous acid (HSNO), which is best described as a mixture

of rapidly interconverting key intermediate isomers: SNO^- (thionitrite) and perthionitrite (SSNO^-). HSNO is liposoluble and capable of crossing cell membranes and causing further *trans*-nitrosation of proteins. It is reactive in different competitive modes, with a half-life of a few seconds at pH 7.4 toward the homolytic cleavage of the N–S bond, and can be deprotonated at pH ≈ 10 , giving SNO^- . Moreover, it was proposed that NO^\bullet and sulfide form a network of cascading chemical reactions that generate radical intermediates as well as anionic and uncharged solutes, each with *in vitro* and *in vivo* bioactivity (Figure 2).⁶

The reaction of thiols with NO-related species has also been studied. *S*-nitrosothiols are formed by the reaction of NO^+ with thiols (RSH) and are involved in nitric oxide storage and transport. They undergo *transnitrosation* reactions, in which NO^+ is transferred between different nucleophiles: HSNO can even act as an NO^+ shuttle between proteins in different cellular compartments.⁴ This compound has also been shown to decompose to HNO by unclear pathways. Additionally, HNO can also be produced by reaction of thiols directly with NO^\bullet . In the reaction with cysteine, the first step comprises a proton-coupled nucleophilic attack of the thiol toward NO^\bullet , with an effective bimolecular rate $k_{\text{eff}} = 30 \text{ M}^{-1} \text{ s}^{-1}$.⁷ The direct reaction between NO^\bullet and H_2S has been described as eq 1, and the observed rate constant for $\text{HSNO}^{\bullet-}$ production (eq 1) is $k = 8 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$.⁸



As has already been hinted, in biological systems, metalloproteins are deeply involved in the reactivity of all these small molecules. In this context, their relation with heme proteins, especially relevant for nitric-oxide-related species, is particularly interesting. Although the crosstalk between NO^\bullet and H_2S in the presence of iron complexes has already been addressed,^{3,4,8} in this work, we will focus on describing the biologically relevant reactivity of sulfide and nitrosyl species with hemeprotein models. We will revise the reactivity of inorganic sulfide species and nitric oxide derivatives with synthetic porphyrins acting as heme models, comment on research efforts focused on ternary systems involving Fe porphyrins, sulfide species, and NO^\bullet derivatives, and finally expose our thoughts and perspectives on this promising, albeit complicated, bioinorganic study topic.

■ REACTIVITY OF INORGANIC SULFIDE SPECIES TOWARD IRON HEME PLATFORMS

The coordination chemistry of H_2S and its conjugated base to heme proteins is diverse. The interaction of hemoglobin (Hb) and myoglobin (Mb) with H_2S in the presence of O_2 results in covalent modification of the heme periphery to generate sulfmyoglobin and sulfhemoglobin, respectively.⁹ In the absence of O_2 , on the other hand, it is known that the reaction of H_2S toward some heme proteins can form stable Fe^{III} –sulfide complexes, as in the case of hemoglobin I of *Lucina pectinata* (HbI LP), a clam inhabiting sulfide-rich environments. In this case, the protein binds sulfide and forms a hexacoordinated low-spin complex with a very high affinity constant.¹⁰

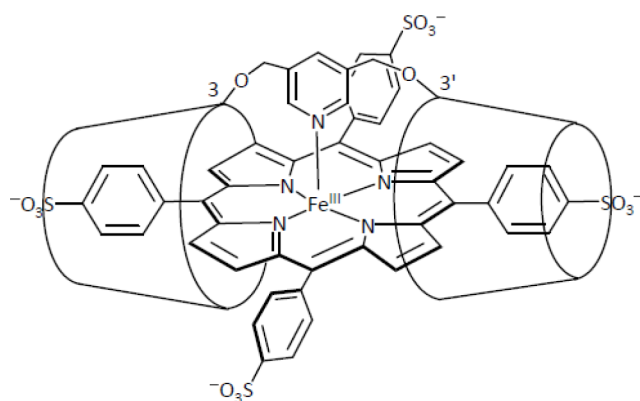


Figure 4. Structure of the $[\text{Fe}^{\text{III}}(\text{TPPS})]^{3-}$ –cyclodextrin inclusion complex (reproduced with permission from ref 15).

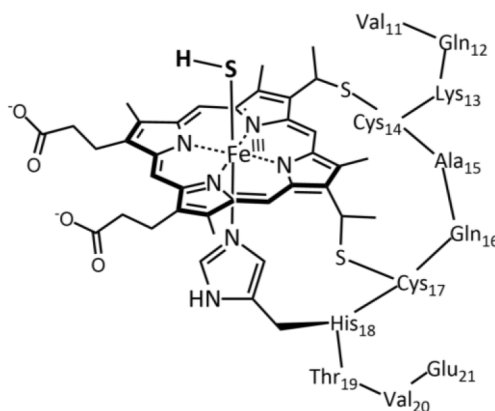


Figure 5. Ferric sulfide complex of microperoxidase 11 (MP11). The numbering of the retained amino acid residues are as in cytochrome c.

Under anaerobic conditions, more cases of sulfide-coordinated heme proteins have been detected and kinetically characterized in buffered aqueous solutions.¹¹ Additionally, it is also known that some heme proteins are reduced by sulfide.⁹ The stabilization of the binding of sulfur species to ferric heme proteins has therefore been widely discussed in terms of the role of neighboring amino acids or other functional structures at the distal and proximal sites, the polarity of the local environment around the heme center, the accessibility of sulfur species to the heme active site, and many variables that cannot be isolated in the protein matrix. To isolate protein factors governing redox chemistry, model compounds are used as representatives of heme proteins. However, biorelevant sulfide coordination chemistry with heme model compounds is scarce, as will be described as follows. In general, free ferric porphyrinates are reduced by inorganic sulfide in aqueous solution, but in certain cases, sulfide binding is favored, as in the example cited in ref 10.

In organic media, ferrous sulfide complexes have been isolated and characterized using iron porphyrinates $[\text{Fe}^{\text{II}}(\text{T-}p\text{-OMePP})]$, $[\text{Fe}^{\text{II}}(\text{OEP})]$, $[\text{Fe}^{\text{II}}(\text{TMP})]$, and $[\text{Fe}^{\text{II}}(\text{TMP})(1\text{-MI})_2]$ (*T-p-OMePP* = 5,10,15,20-Tetrakis(4-methoxyphenyl)porphyrinate; *OEP* = 2,3,7,8,12,13,17,18-Octaethylporphyrinate; *TMP* = 5,10,15,20-Tetrakis(mesityl)porphyrinate; 1-MI = 1-methylimidazole). Strikingly, they resulted in the formation of penta- and hexacoordinated ferrous complexes with general high-spin configurations: $[\text{Fe}^{\text{II}}(\text{Por})(\text{SH})]^-$ and $[\text{Fe}^{\text{II}}(\text{Por})(\text{SH})_2]^{2-}$. The crystal structures of pentacoordinate

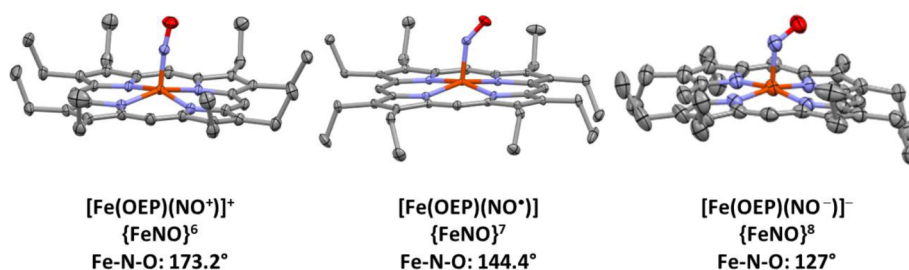


Figure 6. Crystal structures of $\{\text{FeNO}\}^n$ porphyrin complexes of OEP with $n = 6-8$.¹⁸

$[\text{Fe}^{\text{II}}(\text{OEP})(\text{SH})]^-$ and $[\text{Fe}^{\text{II}}(\text{T-}p\text{-OMePP})(\text{SH})]^-$ complexes were obtained, as well as that of the mixed-ligand species $[\text{Fe}^{\text{II}}(\text{Por})(\text{Im})(\text{SH})]^-$. On the other hand, the attempt to obtain ferric complexes was not successful since sulfide reduced all ferric porphyrinates, resulting in the same products observed with ferrous porphyrins.¹² However, a low-spin Fe(III)-SH intermediate could be recently identified during the reduction of ferric porphyrins by sulfide at low temperature in dichloromethane, providing early evidence of this kind of compounds.¹³

The reactivity of the hydrosulfide ion HS^- toward several synthetic ferrous *meso*-tetraphenylporphine (TPP) derivatives has been examined. In all cases, HS^- was found to bind to five-coordinate high-spin ferrous centers following a 1:1 stoichiometry to give $[\text{Fe}^{\text{II}}(\text{SH})(\text{Por})]^-$ complexes. Moreover, an intermediate complex proposed as the hydrosulfide bridged dimer, $(\text{Bu}_4\text{N})[\text{Fe}^{\text{II}}_2(\mu\text{-SH})(\text{F}_8\text{TPP})_2]^-$ ($\text{F}_8\text{TPP} = 5,10,15,20$ -Tetrakis(2,6-difluorophenyl)porphyrinate), was identified by NMR spectroscopy in route to the formation of $(\text{Bu}_4\text{N})[\text{Fe}^{\text{II}}(\text{F}_8\text{TPP})(\text{SH})]^-$ (Figure 3d). Treatment of $(\text{Bu}_4\text{N})[\text{Fe}^{\text{II}}(\text{F}_8\text{TPP})(\text{SH})]^-$ with NO^{\bullet} and 1,2-dimethylimidazole resulted in displacement of the HS^- ligand.¹⁴

In a further study, a *picket-fence porphyrin* model (TPivPP, (All-*cis*)-5,10,15,20-tetrakis[2-(2,2-dimethylpropionamido)-phenyl]porphyrinate, Figure 3f) was used, and it was found that the sterically restricted porphyrin does not bind the neutral H_2S species in either the ferric or ferrous state. In contrast, reaction of $\text{Fe}^{\text{III}}(\text{P})$ with HS^- reduces iron and eventually binds to the ferrous forms.¹⁵ The sulfide binding affinities were similar to those obtained with unhindered porphyrins, and so they concluded that the extra stabilization is related to kinetic rather than thermodynamic effects. The authors also mention the significance of hydrogen bonding interactions in facilitating reversible interaction between sulfide and ferric hemes. All mentioned porphyrin hydrosulfide complexes are shown in Figure 3.

Not many examples in aqueous solution are found in the literature. A very interesting study employs a simple supra-molecular model of hemoglobin composed of the 1:1 inclusion complex of ferric porphyrin $[\text{Fe}^{\text{III}}(\text{TPPS})]^{3-}$ (TPPS = 5,10,15,20-Tetrakis(4-sulfonatophenyl)porphyrinate) in cyclodextrin (CD), linked by a pyridine moiety which acts as a proximal ligand (Figure 4). The reaction of this model compound with HS^- in an oxygen-free solution yields an unusually stable ferric sulfide porphyrin complex. Moreover, the ferric sulfide complex can be generated by exposing the $\text{Fe}(\text{II})-\text{O}_2$ complex to HS^- , via O_2^-/HS^- exchange.¹⁶ Reaction of HS^- with the pyridine-free model yielded the ferrous complex, indicating that reduction occurs instead of coordination.

Hydrosulfide binding to semisynthetic heme systems has been investigated using bis-*N*-acetyl-microperoxidase undecapeptide (NACMP11), a heme peptide derived from cytochrome *c* proteolysis that retains proximal histidine bound to Fe(III). Addition of H_2S to a pH 6.8 solution of $\text{Fe}^{\text{III}}(\text{NACMP11})$ revealed the formation of a moderately stable low-spin ferric sulfide complex. The results suggest that delayed metal reduction is possible with the assistance of proximal mechanisms as a minimum requirement.¹⁷ A joint study combining experimental and theoretical tools showed that both H_2S and HS^- species are capable of binding in the model system $\text{Fe}^{\text{III}}(\text{MP11})$, where access to the active site is only restricted by the presence of a water molecule, and in $\text{Fe}^{\text{III}}(\text{Mb})$ where the access of ligands to the active site is restricted by the topology of the protein matrix. HS^- binding is favored over H_2S , if the active site is accessible (Figure 5).¹²

■ $\{\text{FeNO}\}^n$ PORPHYRIN COMPLEXES: NO^+ , NO^0 , AND NO^-/HNO

Due to their biological significance as models for heme enzymes of the nitrogen cycle, Fe-NO porphyrin complexes are

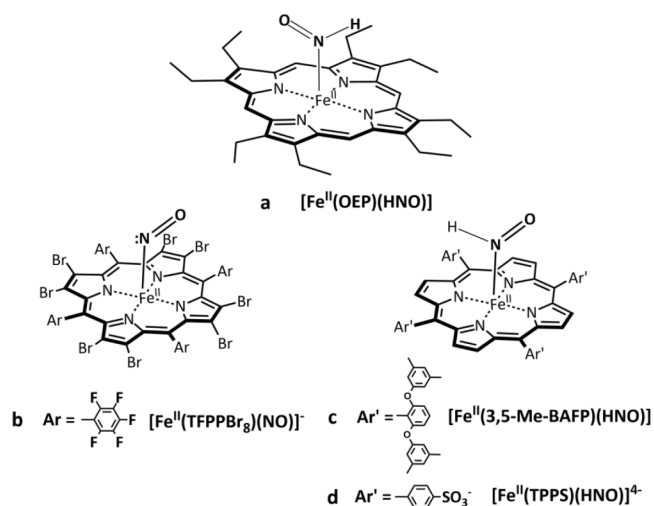


Figure 7. $\{\text{FeHNO}\}^8$ and $\{\text{FeNO}\}^8$ porphyrin complexes.

of high interest, and have been thoroughly studied over the years.¹⁸ Since the NO ligand is redox active and *noninnocent*, it is not always easy to establish the oxidation states involved, and so these complexes can be more conveniently represented as $\{\text{FeNO}\}^n$ using the Enemark-Feltham notation. Here, the Fe-NO fragment is considered as a covalent unit with n electrons, corresponding to the sum of electrons present in the metal's d orbitals and the π^* orbital of the NO ligand.¹⁸ For iron complexes, the limiting descriptions of $\{\text{FeNO}\}^6$,

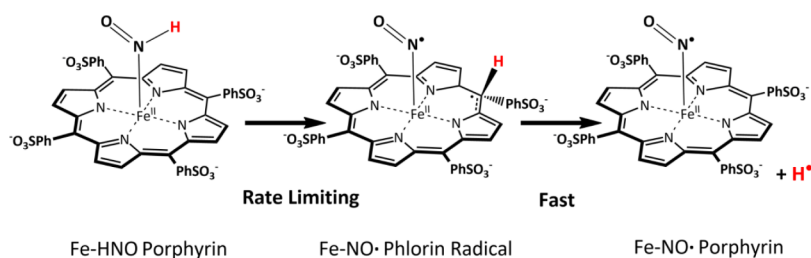


Figure 8. Proposed mechanism for the decomposition of the $\{\text{FeHNO}\}^8$ of TPPS.

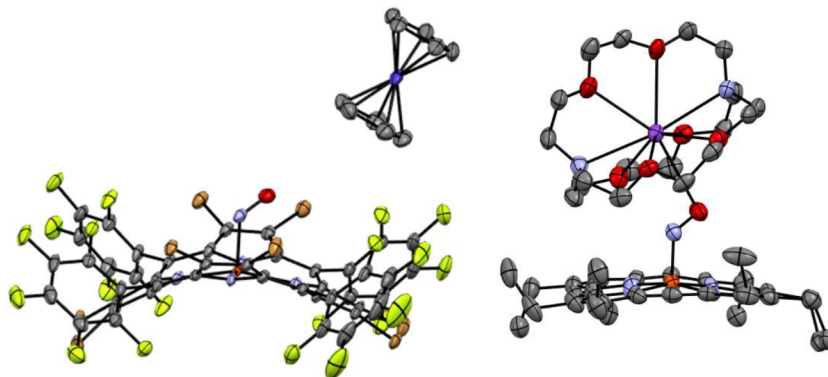


Figure 9. ORTEP diagrams of the crystal structures of $(\text{Co}(\text{Cp})_2)[\text{Fe}(\text{TFPPBr}_8)(\text{NO}^-)]$ (left) and $(\text{K}(2.2.2))[\text{Fe}(\text{OEP})(\text{NO}^-)]$ (2.2.2 cryptand = 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]-hexacosane) (right).¹⁸

$\{\text{FeNO}\}^7$ and $\{\text{FeNO}\}^8$ complexes can be interpreted mainly as Fe(II) coordinated to NO^+ , NO^\bullet and NO^- , respectively, although intermediate electronic distributions are also possible. Fe(II)–HNO complexes, in this context, also with $n = 8$, may be represented as $\{\text{FeHNO}\}^8$. This notation is particularly useful because it allows us to predict the Fe–N–O angle merely as a function of n , as shown in Figure 6 for the $\{\text{FeNO}\}^{6/7/8}$ complexes of Fe^{II}(OEP).¹⁸

$\{\text{FeNO}\}^6$ Porphyrin Complexes. $\{\text{FeNO}\}^6$ complexes are biologically relevant since they constitute reactive intermediates in the enzymatic mechanisms of different proteins such as nitrophorins and P450_{nor}.¹⁸ These complexes show almost linear Fe–N–O angles and are preferentially described as Fe(II)– NO^+ species. This makes the NO fragment electrophilic and susceptible to nucleophilic attack by hydroxide, hydride, alkyl groups, and thiols. Historically, synthetic complexes of this kind were considered kinetically unstable, as they tend to lose NO^\bullet to give free ferric porphyrinates much faster than protein Fe(II)– NO^+ species. However, it has recently been established that the observed instability is caused by halide traces or other kind of impurities and is not an intrinsic property of these complexes.¹⁸ In this context, the electrophilic reactivity of $\{\text{FeNO}\}^6$ porphyrin complexes with physiologically relevant species, such as thiols and other sulfur compounds, is an interesting feature to be explored.

In fact, reaction of the hexacoordinated $\{\text{FeNO}\}^6$ porphyrin complex $[\text{Fe}(\text{OEP})(\text{NO})(\text{S-MI})][\text{OTf}]$ (S-MI = 5-methylimidazole, OTf = triflate) with borohydride resulted in the formation of the corresponding $\{\text{FeHNO}\}^8$ complex, making this an interesting strategy for preparing this elusive kind of species, which are described below.¹⁹ Interestingly, if the starting complex is pentacoordinated, hydride binds to the axial position instead.

Another special property of the $\{\text{FeNO}\}^6$ species is the lack of the so-called trans labilization effect, and so the Fe–L

distance in hexacoordinated $[\text{Fe}(\text{Porf})(\text{NO})(\text{L})]^+$ is very similar to the corresponding $[\text{Fe}(\text{Porf})(\text{L})_2]^+$ complex.¹⁷ This is not the case for $\{\text{FeNO}\}^7$ and $\{\text{FeNO}\}^8$ analogues.²⁰

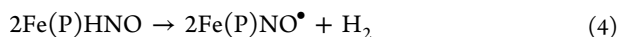
$\{\text{FeNO}\}^7$ Porphyrin Complexes. $\{\text{FeNO}\}^7$ porphyrin complexes are by far the most robust and most explored of the FeNO family. These complexes are paramagnetic and usually described as Fe(II)– NO^\bullet entities. Although most of these compounds are air sensitive, in contrast to $\{\text{FeNO}\}^6$ species, $\{\text{FeNO}\}^7$ porphyrin complexes are more stable toward NO^\bullet loss, with k_{ON} and k_{OFF} in the range of $10^9 \text{ M}^{-1} \text{ s}^{-1}$ and 10^{-4} s^{-1} , respectively.

This reactivity is key for biologically relevant mechanisms, such as the fast activation of sGC. In this process, binding to NO^\bullet promotes the labilization of a proximal histidine, which in turn activates the enzymatic response. These complexes are common intermediates in enzymatic mechanisms of the nitrogen cycle.²¹ Their general stability means that these species have to be activated, by a Lewis acid or via radical–radical coupling, for example, in order to be reactive in physiological conditions.¹⁸ $\{\text{FeNO}\}^6$ and $\{\text{FeNO}\}^7$ porphyrin complexes have been the subject of numerous studies and reviews, and the focus will be put on the less explored $\{\text{FeNO}\}^8$ and $\{\text{FeHNO}\}^8$ species.

$\{\text{FeNO}\}^8$ and $\{\text{FeHNO}\}^8$ Porphyrin Complexes. As the biological activity of nitroxyl, the reduced form of NO^\bullet , became known, the interest of bioinorganic chemists in characterizing and studying the reactivity of its elusive coordination compounds has significantly increased. However, due to their tendency to reoxidize to their $\{\text{FeNO}\}^7$ counterparts, not many examples have been reported for $\{\text{FeNO}\}^8$ and $\{\text{FeHNO}\}^8$ model complexes (Figure 7). In fact, the most stable $\{\text{FeHNO}\}^8$ complexes have been obtained directly in protein environments, with myoglobin and other globins.²² These species were stable for weeks and allowed

detailed characterization by ^1H and ^{15}N NMR, Raman spectroscopy, and X-ray absorption.

The presence of a protective scaffold is key in preventing the decay of $\{\text{FeHNO}\}^8$ complexes, as shown by the remarkable stability of a sterically hindered *bis-picket fence* porphyrin, shown in Figure 7c, whose half-life extended up to 5 h.²³ A bimolecular reaction with hydrogen production was proposed as the main decomposition pathway of these species (eq 4):



This pathway had been proposed in early works, in which the first $\{\text{FeNO}\}^8$ porphyrin complexes were prepared by electrochemical reduction of the nitrosyl complexes of OEP and TPP.¹⁸ Evidence for the $\{\text{FeHNO}\}^8$ adduct of OEP (Figure 7a) was later obtained in the presence of phenols acting as weak acids,²⁴ while a different preparation scheme involving hydride attack on the corresponding hexacoordinated $\{\text{FeNO}\}^6$ complex could even provide NMR and IR characterization.¹⁹

In 2019, the $\{\text{FeHNO}\}^8$ complex of the water-soluble porphyrin TPPS (Figure 7d) could be obtained by chemical reduction of the $\{\text{FeNO}\}^7$ complex and characterized by UV-vis, decomposing completely only after a few minutes.²⁵ The fact that this species could be observed at room temperature using an unhindered porphyrin suggested that other factors apart from steric protection are relevant in the stabilization of these complexes, such as the ability to form strong hydrogen bonds with water. Interestingly, the decomposition reaction did not follow the expected bimolecular pathway but was first order in the complex. DFT calculations suggested a new possible decay route involving hydrogen atom migration to a porphyrin *meso*-carbon to form a transient *phlorin* intermediate. This species would rapidly react to give the starting $\{\text{FeNO}\}^7$ complex (Figure 8).

The nonprotonated $\{\text{FeNO}\}^8$ complex could also be obtained, and the pK_a for the coordinated HNO could be estimated as 9.7 from spectroscopic and electrochemical experiments.

The first successful isolation of a $\{\text{FeNO}\}^8$ complex, on the other hand, was achieved using the electron-poor porphyrinate TFPPBr₈ (5,10,15,20-Tetrakis(pentafluorophenyl)-2,3,7,8,12,13,17,18-Octabromo-porphyrinate) by chemical reduction in organic media (Figure 7b).²⁶ Although this species was highly stabilized by the electron-withdrawing substituents, protonation of the nitroxyl moiety resulted once again in the original $\{\text{FeNO}\}^7$ precursor. This compound and $[\text{Fe}^{\text{II}}(\text{OEP})\text{-(NO}^-)]$ are the only structurally characterized heme $\{\text{FeNO}\}^8$ complexes to date and are shown in Figure 9.²⁷ Although $\{\text{FeNO}\}^8$ complexes are more *trans*-labilizing than $\{\text{FeNO}\}^7$ species, protonation of this fragment virtually eliminates this effect.¹⁸

NO-SULFUR INTERPLAY WITH FE-PORPHYRINS

In the context of biologically relevant interactions, porphyrin complexes of NO^\bullet (or sibling molecules) and sulfur species are of special relevance. The RS-Fe-NO arrangement is commonly found since many heme proteins involved in the nitrogen cycle rely on thiols as the sixth axial ligand.²⁸ Nitric oxide synthase, the enzyme responsible for NO^\bullet biosynthesis, shows a deprotonated cysteine in the proximal position, which also binds the heme site to the protein structure. The same scheme is observed in nitric oxide reductase P450Nor (which

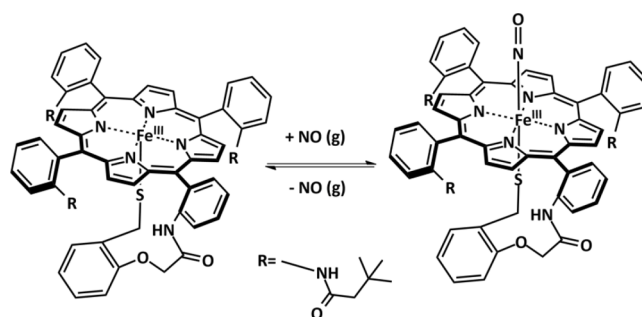


Figure 10. $\text{NO}(\text{g})$ reactivity toward a heme model complex with a covalently linked thiolate moiety.

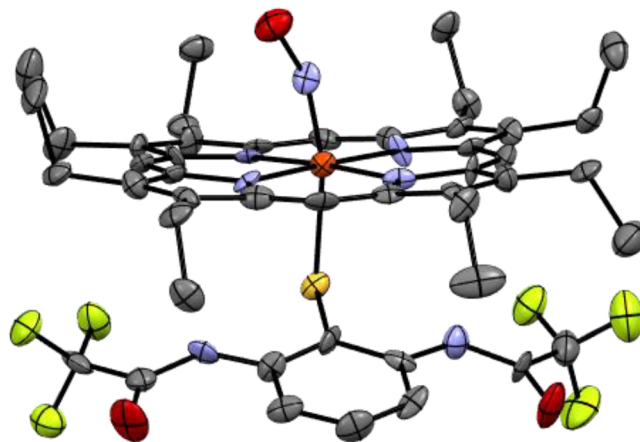


Figure 11. ORTEP structure for the only crystallographically characterized $\{\text{FeNO}\}^6$ heme thiolate complex.³¹

reduces NO^\bullet to N_2O) and other enzymes including certain kinds of nitrite reductase and nitrophorins.

This is no coincidence since coordination to electron-rich sulfur species allows stabilization of oxidated iron states and subsequent substrate activation via a *push effect*. For example, during the enzymatic NOS production of NO^\bullet from arginine, the proposed $\text{Fe}(\text{IV})\text{-O}$ highly oxidizing intermediate is stabilized by electron donation from the cysteine ligand. A similar situation is seen in the enzymatic cycle of P450Nor, in which an alleged $\text{Fe}(\text{IV})\text{-NHOH}$ key intermediate is formed from an $\text{Fe}(\text{II})\text{-HNO}$ complex. The double protonation of this complex can be achieved because of the enhanced basicity of the heme ligand, again due to the electron-donating ability of the *trans*-thiolate residue.²⁰ Moreover, in these complicated protein systems, linkage to thiolate species is found to be stabilized by hydrogen bonding interactions with neighboring amino acids, which also play protective roles and are central to fine-tuning their reactivity.

Given all this context, the study of RS-Fe-NO porphyrin model systems arises as a very valuable tool for examining the influence of all these factors in enzyme reactivity and broadening our understanding of the underlying mechanisms through inorganic chemistry techniques. In laboratory conditions, these systems are elusive, unstable, and not easy to characterize, and so there are not many examples in the literature. Although a few protein $\{\text{FeNO}\}^6$ thiolate-bound species could be prepared,²⁹ model complexes remained elusive.

The reactivity toward $\text{NO}^\bullet(\text{g})$ of a ferric heme thiolate complex, in which the thiol moiety is covalently linked to

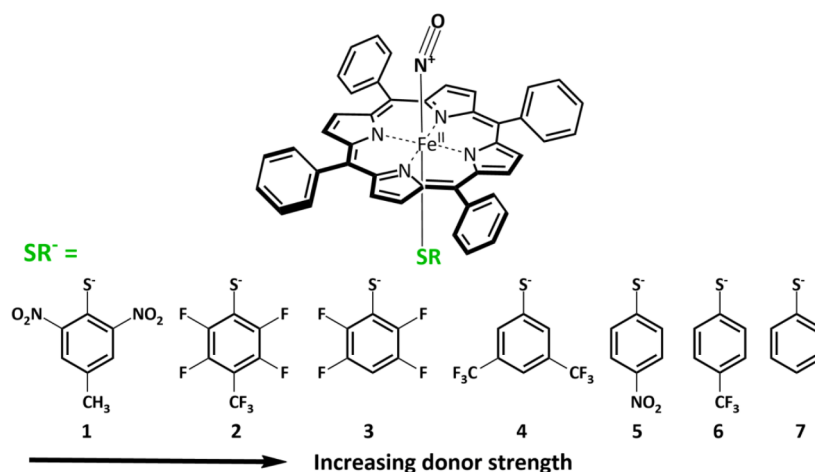


Figure 12. Heme-thiolate $\{\text{FeNO}\}^6$ porphyrin complexes studied by Lehnert and co-workers.¹⁸

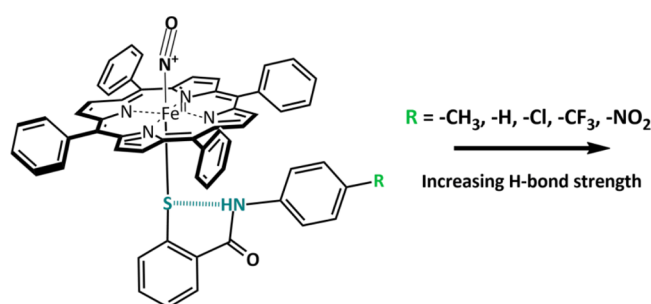


Figure 13. Amide-based heme thiolate complexes with intramolecular hydrogen bonding properties.³²

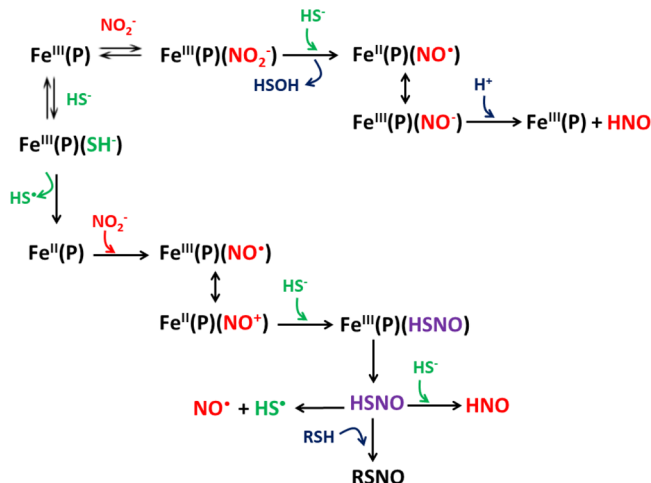


Figure 14. Reaction scheme of the heme-catalyzed nitrite reduction in the presence of HS^- .

porphyrin substituents and protected by bulky groups, was the first to be explored (Figure 10).³⁰ The resulting $\{\text{FeNO}\}^6$ complex was characterized by UV-vis and EPR, and its conversion to the $\{\text{FeNO}\}^7$ form could be achieved by sodium borohydride reduction. NO^\bullet binding was reversible, and the reaction rate depended on the coordinating nature of the solvent, three times faster in toluene than in methanol, where a coordinated solvent molecule needs to be removed. This behavior correlates well with the reactivity observed for heme protein CytP450Cam and emphasizes the importance of the

fine-tuning ability of the protein environment, which can determine whether a vacant position will be available or not.

A bent $\{\text{FeNO}\}^6$ thiolate complex was the first—and yet, only—of its kind to be structurally characterized by reaction of crystalline $\text{Fe}^{\text{III}}(\text{OEP})(\text{thiolate})$ with NO^\bullet gas (Figure 11). In solution, however, reaction led to the formation of the corresponding $\{\text{FeNO}\}^7$ complex and free thiolate.³¹ Interestingly, hydrogen bonding between the thiolate sulfur and neighboring hydrogen atoms is evidenced: a very similar situation is observed in crystalline P450Nor and NOS, in which the $\text{Fe}-\text{S}-\text{R}$ bond is stabilized by hydrogen bonding between the sulfur atom and HN moieties from nearby amide peptide groups and tryptophan residues, respectively.

Considering that a $\{\text{FeNO}\}^6$ complex is formed in the first step of the CytP450Nor enzyme, Lehnert and co-workers studied the reaction of different heme thiolate complexes with NO^\bullet gas in the hope of better understanding the functioning of this naturally occurring system and methodically evaluating the influence of the thiolate ligand.¹⁸ In 2019, they reported the UV-vis characterization of a series of $\{\text{FeNO}\}^6$ complexes prepared by reaction of the corresponding heme thiolate precursors with nitric oxide, only achievable at very low temperatures (Figure 12). Notably, for thiols (1), (2), and (3), the spectral features of the products were similar to those observed in protein complexes. At -80°C , the complexes were stable for more than 2 h, but the authors observed that above -60°C the complexes inevitably start decomposing to give pentacoordinate $\{\text{FeNO}\}^7$. The syntheses of the precursor $[\text{Fe}(\text{TPP})(\text{SR})]$ complexes are also described in their work.

The electron-poor thiophenolates shown in Figure 12 were initially selected via DFT analysis as the best models for the local electronic structure of CytP450nor and were used as axial donors for FeTPP . Their reactivity proved to be sensitive to the electron-donating differences throughout the series. For instance, when thiols (4), (5), and (6) within this group were employed, NO^\bullet was also observed to react directly with the thiol to form nitrosothiols, giving only partial coordination to the Fe center. Not surprisingly, for the most donating thiol (7), this was the only observed outcome.

The complexes and their decomposition products were further characterized by FT-IR and Raman spectroscopy, and interestingly, a correlation was found between the donating strength of the thiolate ligands and the $\text{Fe}-\text{NO}$ and $\text{N}-\text{O}$ stretching frequencies in the thiolate $\{\text{FeNO}\}^6$ models. Less

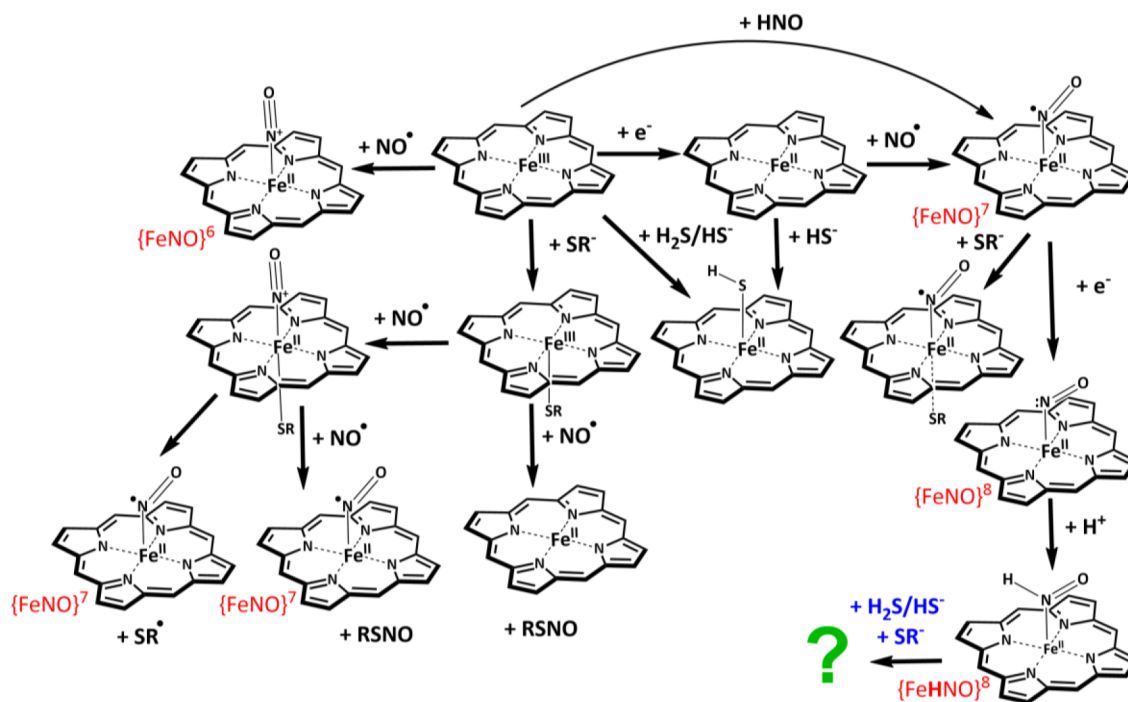


Figure 15. General reaction scheme between iron porphyrins, NO^\bullet redox siblings, and relevant sulfur species.

electron-donating thiols resulted in higher stretching frequencies, in agreement with stronger bonds. This finding confirms the σ -donating effect of the thiol moiety and implies, as previously proposed, that fine-tuning of the electronic environment of heme thiolate proteins results in different degrees of activation of the FeNO fragment. DFT studies also showed that, accordingly, Fe–N–O stretching frequencies can act as probes for establishing the donating strength of a given thiolate ligand.

The effect of the proximal hydrogen bonding network is key since it also influences the donating properties of the thiolate ligands in Nature. This influence was the subject of an additional study with FeTPP, in which the intramolecular H-bond strength was modulated using different amide-based thiolates, shown in Figure 13.³² Again, a correlation was found between H-bond strength and Fe–NO and N–O stretching frequencies: as expected, stronger H bonds resulted in weaker thiolate donation and stronger Fe–NO and N–O bonds (higher vibration frequencies).

The comparison between two thiolates with similar electron-donating properties, but differing in their H-bond availability, showed that the presence of H bonds helps stabilize the thiol moiety toward reactivity with other small molecules (such as NO^\bullet , which would result in nitrosothiol formation) and protonation. Moreover, the effect of multiple weak H bonds—as can be found in a proximal protein environment—turns out to be comparable to the effect of a single stronger bond.

The reaction between $\{\text{FeNO}\}^7$ complexes and thiolates is even less explored. The formation of a weak Fe–S bond (as compared to Fe–N bonds for imidazole-like axial ligands) was evidenced after the reaction of the ferrous $\{\text{FeNO}\}^7$ model Fe(TPP)NO with both thiolates and thioethers, only at low temperature.³³ The resulting complexes could be characterized by EPR spectroscopy and showed a more pronounced radical character of the NO^\bullet fragment. The tetrahydrothiophene

(THT) complex could be characterized by FT-IR and UV–vis spectroscopy at 183 K in solution and in the solid state.

There are no reports on the reactivity of sulfide species toward porphyrin $\{\text{FeNO}\}^8$ and $\{\text{FeHNO}\}^8$ complexes. However, it has been shown that iron porphyrins mediate nitrite reduction to HNO in the presence of H_2S , in aqueous media.³⁴ In a later study, NO^\bullet and HNO production was verified in mitochondria (rich in heme proteins) by incubation with nitrite and H_2S .⁴ The reaction of a water-soluble ferric porphyrin with varying ratios of sulfide and nitrite was evaluated, resulting in the formation of $\{\text{FeNO}\}^7$ complexes, evidencing nitrite reductase activity from the iron porphyrin. This implied the formation of a ferrous porphyrin complex, which could be detected when $\text{H}_2\text{S}/\text{NO}_2^-$ was 2:1, and sulfide acts as the reducing agent. When the ratio was increased to 5:1, an intermediate ferric Fe(P)(SH) complex was detected by EPR, along with HS^\bullet formation from its decomposition to give again the ferrous porphyrin with the catalytic properties. When sulfide was present in 10-fold excess over nitrite (as physiological conditions are thought to be), HNO and N_2O were also detected, making H_2S a strong candidate for being responsible for endogenous nitroxyl generation from nitrite reduction.

Most remarkably, when sulfide was added to the ferric nitrosyl (formed from the reaction of the ferric porphyrin with nitrite), a reaction intermediate was detected, whose identity could be assigned as a rare Fe–HSNO complex by mass spectrometry at -20°C . This again evidences the possibility of a nucleophilic attack by a sulfide species on the electrophilic $\{\text{FeNO}\}^6$ fragment and posed a probable biosynthetic pathway for the strong nitrosating agent HSNO. The authors conclude their investigation by showing the generation of S-nitrosothiols in cells incubated with nitrite and hydrogen sulfide. A reaction scheme for all the involved processes is shown in Figure 14, which is adapted from ref 4.

PERSPECTIVES

The overall complex reactivity between iron porphyrins, NO-related species, thiols, and hydrogen sulfide described throughout this work is represented in Figure 15. The chemistry involving these species is often particularly challenging since it involves the generation and manipulation of reactive and sensitive species, making their study not a straightforward task.

Although a great deal of progress has been achieved in this subject during the last decades, these systems are far from being understood, and many reactions remain to be studied. For example, there are very few examples of the reactivity between {FeNO}⁷ porphyrin complexes and sulfur species in general, and in particular, there are almost no reports exploring the reactivity of water-soluble nitrosylated porphyrins with H₂S, HS⁻, and thiols.

In this regard, the reactivity of the elusive {FeNO}⁸ and {FeHNO}⁸ nitroxyl porphyrin complexes toward hydrogen sulfide and related species is a completely unexplored subject. Taking advantage of the relative stabilization of these complexes in water using the [Fe(TPPS)]³⁻ complex, the study of their reactivity toward biologically relevant sulfur species arises as an exciting challenge which we are committed to undertake in the near future in our laboratory.

Probably, a good starting point would be to study the reaction between sulfides and iron porphyrins coordinated to nitroxyl. Also, isolation of the RSNO/porphyrin compounds and the study of their reactions and decomposition products will certainly give important data to understand the elusive {FeNO}⁸/RSH chemistry.

All in all, the rich interplay seen in solution and gas studies between NO and sulfur species shows a promising platform for research which will remain fruitful for years to come.

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Notes

The authors declare no competing financial interest.

Biographies

Dr. Silvina Bieza was born in Catamarca, Argentina. She obtained her Ph.D. in chemistry from the University of Buenos Aires in 2020, focused on the reactivity of inorganic sulfide species toward a heme protein model. She is currently completing her postdoc focused on organometallic and bioinorganic chemistry of iron NO, HNO, and H₂S porphyrin complexes with Dr. Prof. Doctorovich.

Agostina M. Mazzeo was born in Buenos Aires, Argentina, in 1991. She obtained her M.Sc. in chemistry from the University of Buenos Aires in 2015, where she works as a teaching assistant. She is currently completing her Ph.D. thesis focused on organometallic and bioinorganic chemistry of iron NO and HNO porphyrin complexes under the supervision of Dr. Pellegrino and Prof. Doctorovich.

Dr. Juan Pellegrino received his Ph.D. in chemistry in 2013 at the University of Buenos Aires. His doctoral thesis has focused on organometallic and bioinorganic chemistry of NO complexes. After a postdoc stay at Prof. Milstein's lab at the Weizmann Institute of Science, he was established as an assistant researcher at the University of Buenos Aires. His main research interests include the use of transition metal complexes with noninnocent ligands in key chemical transformations, such as bond activation or proton reduction.

Prof. Dr. Fabio Doctorovich, Full Professor at the University of Buenos Aires and Researcher at CONICET, obtained his Ph.D. in Organic Chemistry from the University of Buenos Aires in 1990. He was a postdoctoral fellow at the Georgia Institute of Technology working with Prof. E.C. Ashby and K. Barefield on single electron transfer and on chemical reactions taking place in nuclear waste tanks. Back in Argentina (UBA), he started to work on nitric oxide (NO), including organic nitrosocompounds, inorganic iron, rhodium, ruthenium and iridium nitrosyl complexes, and reactivity of metalloporphyrins and pincer complexes toward HNO. He also worked on CO complexes, catalytic reactions, and other topics. Nowadays, his main research focus is on reactions involving HNO. Prof. Doctorovich has published over 130 works in international journals and coedited the book *The Chemistry and Biology of Nitroxyl (HNO)*. He was awarded a Guggenheim Fellowship in 2011.

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