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NITRIC OXIDE AND NITRITE REDUCTION BY METALLOENZYMES

Radu SILAGHI-DUMITRESCU

"Babeş-Bolyai" University, Department of Chemistry and Chemical Engineering, Arany Janos street 11, Cluj-Napoca RO-400028, Roumania, Fax: +40-264-590818, e-mail: rsilaghi@chem.ubbcluj.ro

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Recent computational results from our group are reviewed on the mechanisms of enzymatic nitrite reduction to nitric oxide or ammonia, as well as nitric oxide reduction to nitrous oxide or ammonia, with some consideration for the electronic structures of electromeric species such as metal-nitric oxide adducts. Metal centers discussed include heme *b*, heme d_1, heme *c*, non-heme diiron, and copper.

Radu Silaghi-Dumitrescu was born in 1974 in Braşov, Roumania. He graduated from the "Babes-Bolyai" University in Cluj-Napoca, Roumania in 1997, with a B. Sc. Degree in chemistry, followed by an M. Sc. degree in heterocycle chemistry in 1998. He joined the faculty of the Department of Chemistry and Chemical Engineering in 1998, as assistant. He obtained a PhD degree in bioinorganic chemistry at the University of Georgia at Athens, GA, USA (2000-2004) under the direction of Prof. Donald M. Kurtz, Jr., working on non-heme iron proteins involved in oxidative and nitrosative stress. In 2005 he obtained a second PhD degree in chemistry under the direction of Prof. Ionel Haiduc, focused on theoretical studies of small molecule activation by hemes. In 2004 he took on a postdoctoral position in England at the University of Essex under the direction of Profs. Chris Cooper and Michael Wilson, working on hemoglobin-based blood substitutes. In 2006 he returned to the Department of Chemistry in Cluj-Napoca, where he became an associate professor in 2007, teaching bioinorganic chemistry and biochemistry. His current research interests are small molecule activation by metalloproteins and related metal centers, as studied by multidisciplinary approaches. The ISI database indexes 36 of his publications, cited a total of 158 times (self-citations excluded).

INTRODUCTION

Under anaerobic conditions, many microorganisms can sustain growth by using nitrate as respiratory terminal electron acceptor. Bacteria in fact exhibit a wide range of metabolic reactions with various oxides of nitrogen, sometimes referred to as the bacterial nitrogen cycle.[1] Nitrate and nitrite may be reduced to ammonia for the purpose of nitrogen assimilation (incorporation into organic matter, non-energy conserving) or dissimilation (using nitrate as respiratory electron acceptor, *i.e.*, energy-conserving, but without incorporating the final reduced product into organic matter). Reduction of dinitrogen to ammonia is termed nitrogen fixation. All the known enzymes catalyzing the reactions of the nitrogen cycle are metalloenzymes. Nitrate reduction to nitrite is catalyzed by nitrate reductases, which are molybdopterin enzymes. The subsequent reduction of nitrite is catalyzed by two types of nitrite reductases: those reducing nitrite to ammonia (cytochrome c nitrite reductase, siroheme-containing nitrite reductase) and those reducing nitrite to nitric oxide (copper-containing nitrite reductase, cytochrome cd_1 nitrite reductase).[2-5] When produced by an NO-forming nitrite reductase, nitric oxide is further reduced to N_2O by nitric oxide reductases, which contain either cytochrome bd-or P450-type active sites;[3] non-heme

nitric oxide reductases have also recently been described.[6,7] Nitrous oxide (a relatively inert gas) may either be released as end-product or further reduced to molecular nitrogen by nitrous oxide reductases (which are multinuclear copper proteins). Molecular nitrogen is reduced to ammonia by nitrogenases. Thus, biological nitrate reduction leads to one of three end-products: ammonia (which may be readily incorporated into organic matter), nitrous oxide, or dinitrogen. Additionally, ANAMOX, a little understood biological process, is proposed to generate molecular nitrogen from nitrate and ammonia via hydroxylamine and hydrazine.[1]

Nitrification is an oxidative pathway starting from ammonia and also generates partially reduced nitrogen oxides. Thus, oxidation of ammonia to hydroxylamine is catalyzed by ammonia monooxygenase (a little understood enzyme, apparently related to the particulate methane monooxygenase), and is followed by oxidation of hydroxylamine to nitrite by hydroxylamine oxidase (a multiheme enzyme). These oxidations provide electrons necessary for respiratory electron transfer chains that facilitate lithotrophic growth.[1] In addition to the above described nitrogen cycle, reduction of nitrite to nitric oxide has also recently been proposed to be an important secondary function of hemoglobin in humans, whereby the vasodilator molecule nitric oxide (of Nobel Prize fame) would be generated.[8]

NITRIC OXIDE REDUCTION AND THE AXIAL "PUSH EFFECT"

The active site of fungal nitric oxide reductases (enzymes that belong to the superfamily of cytochromes P450, and are commonly referred to as P450nor) consists of a solvent-exposed, cysteinate-ligated heme.[9,10] Initially proposed mechanisms of P450nor (Figure 1) involved binding of NO to the ferric form of the active site heme, followed by reduction of this $[FeNO]^6$ (in Enemark-Feltham formalism)[11] complex by NAD(P)H. Subsequent attack of a second molecule of NO leads to formation of a nitrogen-nitrogen bond and liberation of the very stable N_2O. Besides the starting $[FeNO]^6$ species, only one intermediate, absorbing at 444 nm, has been experimentally observed.[12]

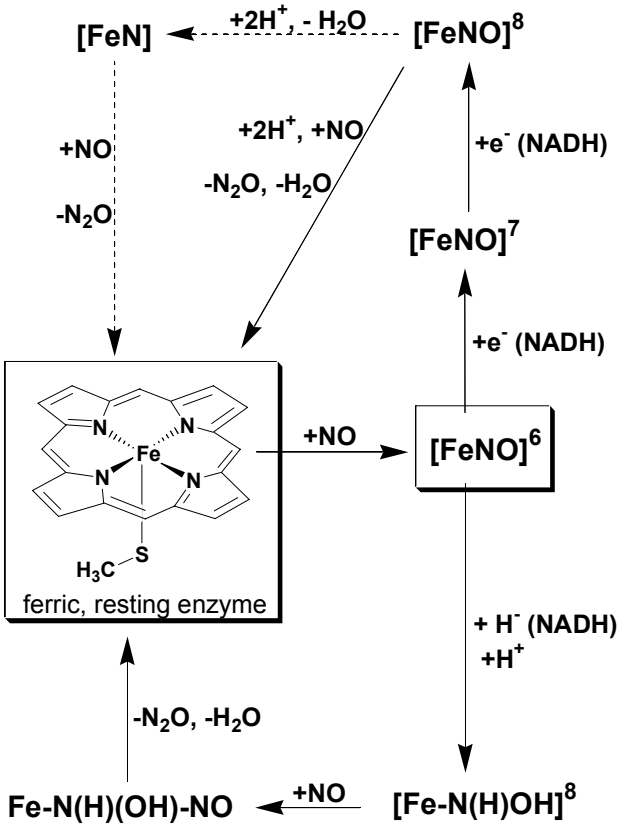


Fig. 1 – Two proposed mechanisms for P450nor.[3] Species unambiguously known to be involved in the catalytic cycle are highlighted. Species in the top and bottom right corners, respectively, have been proposed to constitute an experimentally-observed "444-nm intermediate".[2,13] Marked in dashed arrows is an alternative, less likely pathway.[13]

The details of the P450nor mechanism, and in particular the identity of the 444-nm intermediate, were for a while controversial. One theory[14] (top half, Figure 1), supported by computational results,[13] assumed two sequential one-electron transfers from NAD(P)H to the initial $[FeNO]^6$ complex. The $[FeNO]^8$ species thus formed would constitute the 444-nm intermediate, and would react with NO, eventually liberating the unstable $ONNO^{2-}$ anion (most probably in its protonated form), which decomposes to N_2O and water. However, more recent experimental results[15] support a theory originally put forth by Averill[16], suggesting the first committed step of the mechanism to be direct hydride transfer from NAD(P)H to the nitrogen atom of $[FeNO]^6$, resulting in an iron-bound HNO unit, $[Fe\text{-}(H)NO]^8$, that would undergo protonation to $[Fe\text{-}(H)NOH]^8$ (444-nm intermediate). Subsequent NO addition would yield the unstable HO-N(H)-N=O, which would dissociate from the heme and decompose to H_2O and N_2O.[3]

According to the hypothesis that two electrons might be sequentially delivered to the active-site $[FeNO]^6$ complex by NADH in an outer-sphere mechanism, to generate $[FeNO]^8$ (which would be the experimentally-observed 444-nm intermediate), any one-electron reducing agent with a redox potential equal to or lower than that of NADH, should yield the same $[FeNO]^8$ intermediate upon reaction with the $[FeNO]^6$ at the P450nor active site. Yet, this is known not to be the case: dithionite (redox potential lower than that of NADH by ~100 mV) can only reduce the active site $[FeNO]^6$ to $[FeNO]^7$.[12] Daiber *et al.*[15] have pointed out this shortcoming of the one-electron theory, and have instead shown kinetic isotope effects, using deuterium-substituted NADH, thereby concluding that hydride transfer from NADH to the nitrogen atom of $[FeNO]^6$ was the key step of the mechanism. The 444-nm intermediate would thus be $[Fe\text{-}N(H)\text{-}OH]^8$, with the second proton provided by solvent and/or protein matrix. In support of this latter interpretation, we have computed energies for $[FeNO]^6$, $[FeNO]^7$ and $[FeNO]^8$ models of the P450nor active site, suggesting that a mechanism implying two sequential one-electron transfer steps for the reduction of $[FeNO]^6$ to $[FeNO]^7$ to $[FeNO]^8$ would be energetically unfeasible.[3] Thus, a $[FeNO]^7$ species would be generated as an intermediate, that is more than 200 kJ/mol more stable than the end-product $[FeNO]^8$, and some 150 kJ/mol more stable than $[FeNO]^6$. Therefore, with one-electron reducing agents (such as dithionite) the P450nor site remains trapped in a $[FeNO]^7$ form that is too stable to be catalitically competent.[3] By contrast, with a two-electron donor, such as NADH, reduction proceeds from $[FeNO]^6$ *directly* to the (protonated or not) $[FeNO]^8$ stage. This observation also identifies the reason for which the P450nor active site is solvent-exposed and allows NADH access to the heme,[10] unlike in any other member of the P450 protein[17] family. Thus, all other members of the P450 protein family have a heme active site buried inside the protein, and electrons are supplied to this heme by NADH in an indirect, outer-sphere, long-range manner, via flavin moieties, **in two sequential one-electron steps**.[17] Unlike P450nor, these other P450 proteins do not exhibit any nitric oxide reductase activity. Thus, the ability to perform the two-electron oxidation of NO at a heme-thiolate active site seems to depend on the possibility of performing the two-electron reduction **in one single step**.[3]

Cytochrome c nitrite reductase (ccNirR) contains a heme at the active site, which can reduce NO to ammonia (with NO being in fact an intermediate product of nitrite reduction).[2] Electrons for this reduction are supplied by five neighboring heme units within the protein. Nitric oxide reduction by ccNiR may be deemed as unexpected, since no hydride donor is required and the active site heme is actually not ligated by a thiolate, but by a lysine side-chain. Indeed, thiolate ligands are commonly believed to exert a certain "thiolate push effect" that would enable heme actives site to accomplish key reactions such as dioxygen activation or NO reduction. How can ccNiR perform the same chemistry as P450nor in the absence of a hydride donor **and** of a thiolate ligand? A simple answer was provided by our calculations on $[FeNO]^{6\text{-}8}$ models where the thiolate ligand was replaced by a methyl-ammine unit to mimic the ccNiR active-site heme. Unlike in P450nor, the ccNiR $[FeNO]^8$ complex is predicted to be more stable than $Fe[NO]^7$.[3] We proposed this to be the reason why in ccNiR there is no need for a two-electron (hydride) donor. Also according to our calculations, qualitatively the same situation as in the ccNiR models occurs if the axial ligand trans to NO is omitted altogether.[3] A general observation may then be, that weak heme axial ligands (such as

lysine, and, most likely, histidine) favor easier (**thermodinamically speaking**) reduction of NO than stronger (anionic) ligands such as thiolate. We further postulated that the same type of argument may hold true for other heme enzymes, such as oxygen-activating (heme-thiolate) and oxygen-binding (heme-histidine) enzymes.[3] That is, reduction of the Fe(II)-O_2 complex in heme-thiolate enzymes would be endothermic, and therefore amenable to regulation (as is required for this type of reaction), while for a heme-histidine Fe(II)-O_2 complex reduction would be too thermodynamically facile to be a useful, controllable process. We therefore proposed **that the old dogma of the "thiolate push effect" in heme enzymes, whereby thiolate would facilitate certain reactions by pushing electron density onto the iron, be replaced by a vision where the thiolate in fact obstructs these reactions, therefore making them more amenable to the control and selectivity required of them.** On the other hand, geometrical and electronic structure data on Fe-NO and related adducts do clearly confirm that the "thiolate push effect" is a reality. Indeed, in thiolate-ligated systems the electron density on the NO moiety is consistently higher (both charge-wise and spin-wise) than in their non-thiolate counterparts. Nevertheless, the differences are not large enough (especially at the level of Fe-N and N-O distances) to warrant the assumption that this "push effect" significantly weakens the respective bonds to the extent that they would be easier to cleave.[3]

Subsequent data was put forth to show the applicability of the "thiolate obstruction" paradigm on a series of heme $[FeNO]^x$ adducts featuring a histidine axial ligand (as opposed to the thiolate and lysine ligands previously examined).[18] Furthermore, the reduction [Fe(II)-dioxygen]→[Fe(II)-superoxide], central in the mechanisms of enzymes such as cytochrome P450, heme oxygenase and cytochrome oxidase, and also possible in cytochrome cd_1 nitrite reductase, also obeys the same rules, with the thiolate obstructing the reaction rather than favoring it.[19] Generalizing, **for reductive processes involving a small diatomic as the sole substrate, neutral ligands (*e.g.*, histidine in cytochrome oxidases and heme oxygenase, lysine in cytochrome *c* nitrite reductase) are found to always be preferable over anionic ligands. By contrast, in enzymes designed to deal with more than one substrate, anionic ligands are preferable (e.g., cysteine or tyrosine in monooxygenases), since they allow the safety switches needed to avoid uncoupling in their race against entropy.**[3,18,19] To our knowledge, this is the first time that such a general interpretation has been put forth for explaining the choice of axial ligands in hemoproteins.

METAL-NITRIC OXIDE ADDUCTS: ELECTROMERISM AND EXPERIMENTAL CHALLENGES

In cytochrome cd_1 nitrite reductase (NiR), the heme d_1 is the site of nitrite reduction to nitric oxide. The crystal structure of NiR bound to nitric oxide is available. This complex, for which a formal Fe(III)-NO ($[FeNO]^6$, in Enemark-Feltham notation) oxidation state was assumed, featured a bent Fe-N-O unit with a long (2.0 Å) Fe-NO bond, as opposed to the typical linear Fe-N-O moiety with a short (under 1.7 Å) Fe-N bond typically found in Fe(III)-NO heme adducts. Our density functional geometry optimization results on models of the ferrous and ferric-nitrosyl complexes of the NiR active site have, contrary to previous interpretations of the NiR-NO crystal structure, provided no evidence to support an atypical geometry for the Fe(III)-NO unit at the NiR active site. Rather, the crystal structure of the NO-bound NiR was shown to be consistent with an Fe(II)-NO complex distorted by strong hydrogen bonding, with possible contributions from a disordered ligand.[20]

Dioxygen binding to ferrous iron (such as in hemoglobin and myoglobin) has long challenged chemists insofar as the electronic description of the formally ferrous-dioxygen species had remained a subject of controversy, with various forms of ferrous-dioxygen or ferric-superoxo descriptions being considered; related species, such as ferric-peroxo or ferryl, present the same problem.[21-26] Density functional theory (DFT) methods provide a description of either closed-shell singlet (ferrous-dioxygen), or open-shell singlet (ferric-superoxo), depending on the functional employed.[19,27] The controversy still surrounding the physical meaning of DFT-derived molecular orbitals and spin populations, as well as the fact that DFT is a single-reference method, would speak against DFT as a reliable tool for understanding the details of

electronic structure within delicate cases such as the heme "ferrous-dioxygen" adduct. By contrast, state-of-the-art multiconfigurational methods (CASPT2) have shown the electronic structure of the Fe-OO moiety as a mixture of states, with ferrous-dioxygen and ferric-superoxo as main players.[27] These latter findings question the usefulness of DFT methods for understanding the electronic structures of transition metal complexes. On the other hand, one unquestioned advantage of DFT methods is that they provide accurate **geometries**; indeed, even for the above-mentioned multireference study of a ferrous-dioxygen heme complex, a DFT-derived geometry was used.[27] Similarly disputed has been the electronic structure of non-heme iron nitrosyl species, S=3/2 $\{FeNO\}^7$ in Enemark-Feltham notation. Fe(II) + NO• or Fe(III) + NO^- descriptions have been proposed, both supported by DFT-derived spin populations, molecular orbitals, or other properties derived there from.[28-32] The recent emergence of S=3/2 $\{FeNO\}^7$ species as likely catalytic intermediates in the nitrosative stress defense mechanisms of bacteria/pathogens has placed new emphasis on these complexes.[33,34] We have therefore proposed a procedure to address electronic structure problems with DFT methods without resorting to the otherwise controversial calculated molecular orbitals, spin densities, or properties derived there from.[26] The proposed approach focuses on the use of the only DFT-derived parameter that is generally recognized to be reliable: the geometry. We thus examined DFT-derived O-O bond lengths in formally ferrous-dioxygen models, and compared them to bond lengths in free, non metal-bound, dioxygen, superoxide and peroxide moieties.[26] Likewise, we compared the N-O bond lengths within ferrous-nitrosyl $\{FeNO\}^7$ models, with the same parameter in free NO^+, NO•, and HNO species. This allows a calibrated, straightforward way of assigning the electronic structure in systems where electromerism makes detailed single-reference molecular orbital analysis unreliable.[26]

For S=3/2 $\{FeNO\}^7$ complexes a debate exists over the nature of the NO ligand, with S=1/2 NO and S=1 NO^- descriptions favoured in different reports.[28-32] Table 1 lists N-O bond lengths for a selected set of experimentally-known non-heme S=3/2 $\{FeNO\}^7$ species (models shown in Figure 2),[29,35-40] together with reference values for free NO and NO^-/HNO.[26]

Taking the N-O bond lengths of S=1/2 NO (1.164 Å) and S=1 HNO or NO^- (1.238 and 1.293 Å, respectively) as references (with HNO preferable as reference over NO^- for arguments similar to those used in discussing OOH vs. OO^- for Table 1), a distinct heterogeneity can be observed among the complexes examined here: whereas the aqua and Rieske dioxygenase (RDO) models appear to be well-described as featuring an S=1/2 NO ligand, the enzyme models PCD (protocatechuate dioxygenase) and IPNS (isopenicillin synthase) appear as equal mixtures of NO and NO^- character, while the SOR (superoxide reductase) model appears distinctly closer to the NO^- description. As a general observation and in line with expectations, the N-O bond length appears to be controlled by the amount of charge pushed by the ligands onto the iron: models that have either a smaller number of ligands, or no anionic ligands (*i.e.*, RDO and aqua) have shorter bond lengths than models that feature more ligands among which at least one is anionic.[26]

Table 1

Calculated N-O bond lengths (Å). Accurate experimental values[41,42] are listed in parentheses for the small models (not available for S=1 HNO, as the ground state is S=0).Figure 2 shows the structures of the species referenced in this Table.[26]

model	N-O	model	N-O
NO	1.164 (1.151)	$[Fe(PCD)NO]^0$	1.196
S=1 HNO	1.238	$[Fe(IPNS)(NO)]^0$	1.197
S=1 NO^-	1.293 (1.260)	$[Fe(RDO)(NO)]^{+1}$	1.163
$[Fe(EDTA)(NO)]^{-1}$	1.197	$[Fe(SOR)(NO)]^{+1}$	1.224
$[Fe(H_2O)_5(NO)]^{+2}$	1.151	$[Fe(ktgl)(NO)]^0$	1.184

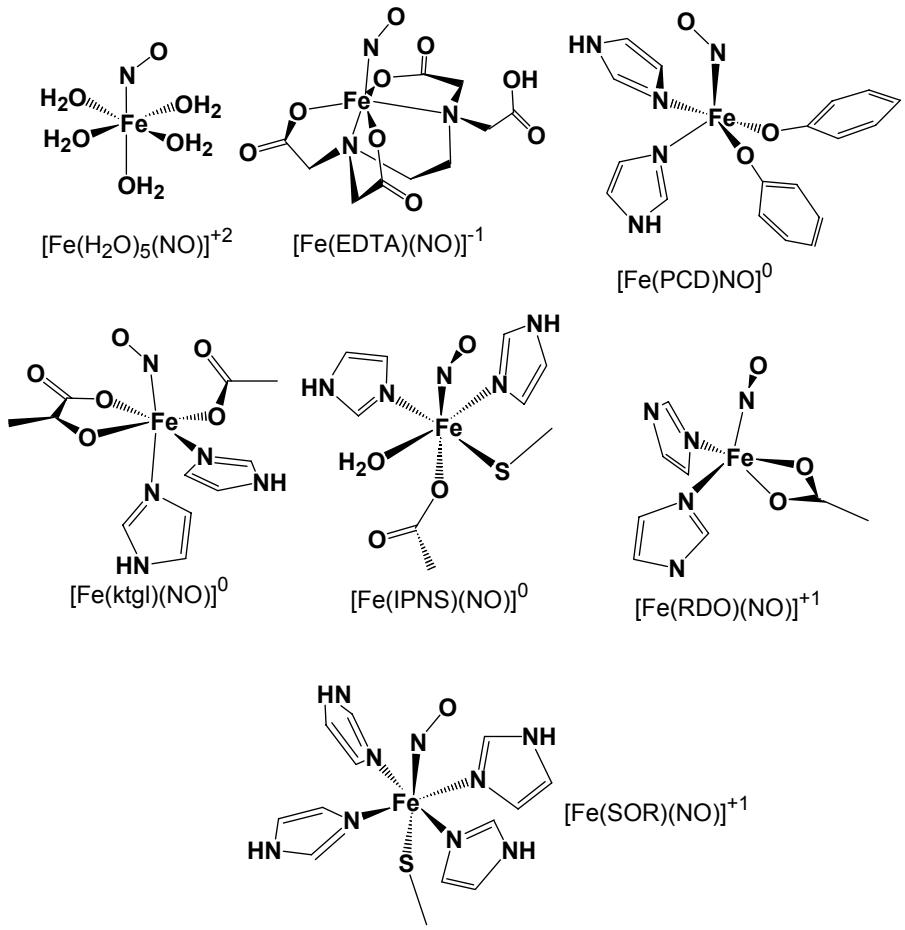
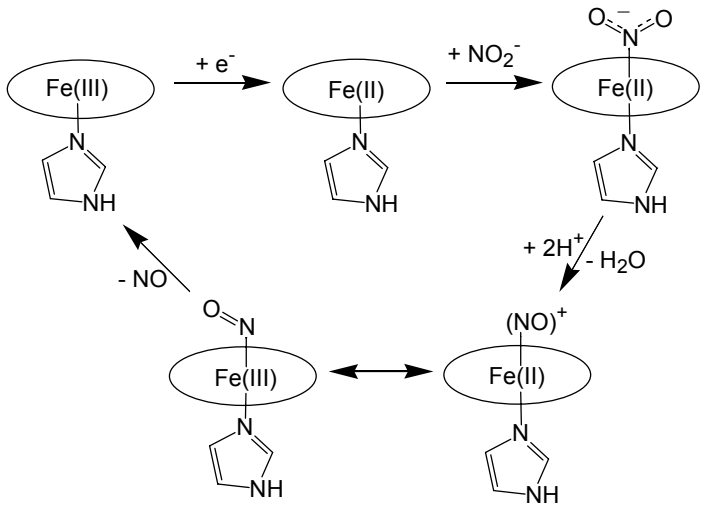


Fig. 2 – S=3/2 $[FeNO]^7$ models employed in the present study. EDTA = ethylenediammino tetraacetate. PCD = model of the active site of protocatechuate dioxygenase, featuring two tyrosine and two histidine ligands. SOR = model of the active site of superoxide reductase, featuring four histidine and one cysteine ligand. ktgl = model of the active site of clavaminate synthase complexed with 2-oxoglutarate and featuring two histidine and one aspartate protein-derived ligands. IPNS = model of the active site of isopenicillin synthase. RDO = model the active site of naphtalene dioxygenase (a Rieske dioxygenase), featuring two histidine and one aspartate ligands.[26]

NITRITE REDUCTION

Nitrite reduction by cytochrome cd_1 nitrite reductase (cd_1NIR) has long been proposed to occur (cf. Scheme 1) via N-coordination of nitrite to the d_1 heme of cd_1NIR. Protonation of a nitrite oxygen atom within the ferrous-nitrite complex would lead to release of a water molecule, forming a weakly-bound complex, that subsequently decays via NO liberation. Nitrite and nitric oxide adducts of the d_1 heme in cd_1NIR have been characterized experimentally and computationally.[4,20,43,44]

Our group has however explored an alternative possibility, involving linkage isomerism of the nitrite at the NIR site.[4] Density functional theory results were thus reported on the previously unexplored O-binding of nitrite to ferrous and ferric cd1NIR. Although the N- isomer (nitro) is energetically favored over the O-nitrite (nitrito), even one single strong hydrogen bond may provide the energy required to put the two isomers on level terms. When hydrogen bonding existent at the cd1NIR active site was accounted for in the computational model, the O-nitrite isomer is found to spontaneously protonate and thus yield a ferric-hydroxo species, liberating nitric oxide. An O-nitrite ferrous cd1NIR complex appears to be an energetically-feasible intermediate for nitrite reduction. O-coordination would offer an advantage since the end-product of nitrite reduction would be a ferric-hydroxo/water complex, rather than the more kinetically inert iron-nitrosyl complex implied by the N-nitrite mechanism. Some of this computational data is illustrated in Figure 3.[4]



Scheme 1

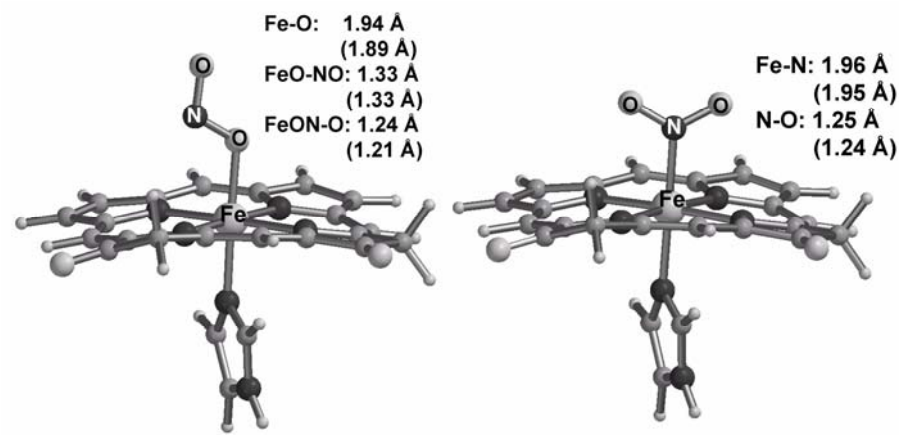
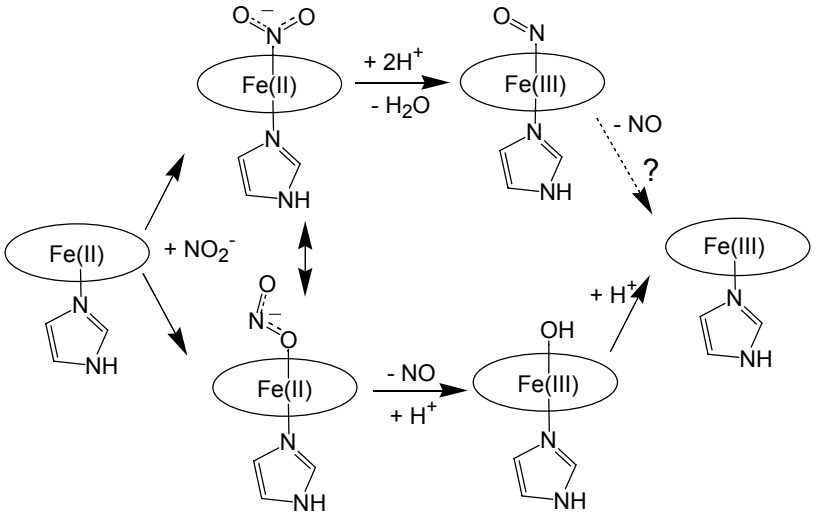


Fig. 3 – Optimized geometries for O- and N- ferrous-nitrite models of the cd_1NIR active site. Distances for the corresponding ferric-nitrite models are given in parentheses [4].

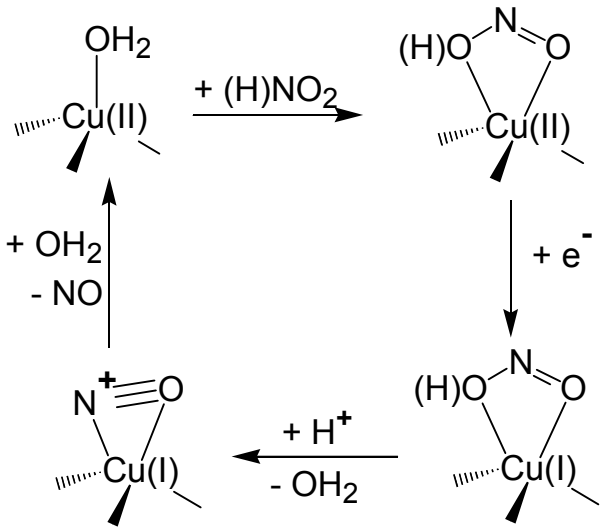
Our revised catalytic cycle for cd1NIR is thus illustrated in Scheme 2. This mechanism, unlike the one in Scheme 1, reconciles for the first time the cd1NIR chemistry with the puzzling fact that Fe(III)-NO is kinetically inert and hence cannot possibly be a part of the cd1NIR catalytic cycle [4].



Scheme 2

The same process as in cd1NIR, i.e. reduction of nitrite to nitric oxide, is also catalyzed by copper-containing NIR (Cu-NIR).[5,45] The proposed catalytic mechanism, illustrated in Scheme 3, has recently been confirmed by our own computational investigations. However, we have found that even for Cu-NIR, nitrite linkage isomerism is an intrinsic property of the metal site, a property which the protein has to modulate in order to achieve its goal of rapid catalytic turnover. Figure 4 illustrates some of our computational results leading to these conclusions.[5]

Independent experimental work has also recently supported the concept that linkage isomerism has a profound influence on the products and mechanisms of catalytic reduction of nitrite by free hemes and related small complexes in solution.[46,47] Thus, the reactions of nitrate and nitrite with sodium dithionite in the presence of Co^{II} tetrasulfophthalocyaninate in aqueous alkaline solution lead to different products (nitrous oxide and ammonia, respectively). These striking differences were explained in terms of different structures of the intermediate complex between Co^{I} phthalocyaninate and substrate, in which nitrite and nitrate were suggested to coordinate via nitrogen and oxygen, respectively. O-coordination of nitrite had also been proved for ruthenium and manganese porphyrinates.[4,46] Perhaps the most compelling experimental evidence in support of O-nitrite isomers in hemoproteins has recently come from Richter-Addo and co-workers,[48] who, upon solving the crystal structure of the myoglobin-nitrite adduct (Figure 5), found only the nitrite isomer to be present – which, while constituting the first direct structural observation of an O-nitrite adduct in a heme protein, was predicted by our computational results two years in advance.



Scheme 3

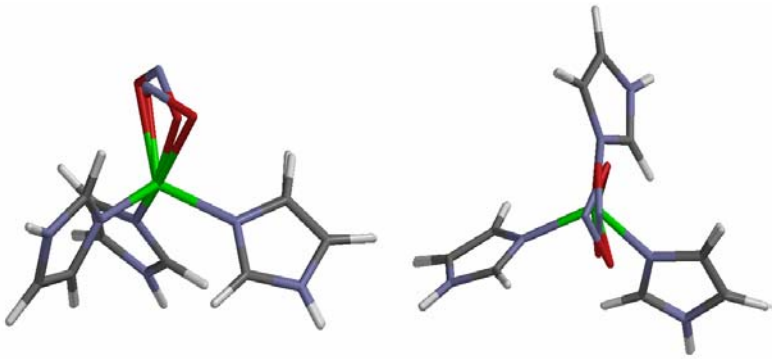
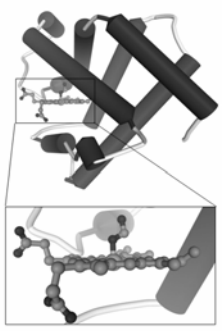


Fig. 4 – Overlay (viewed along the and perpendicular to the approximate Cu-nitrite axis, respectively) of the experimental (pdb code 1SJM) and calculated structures of the Cu(II)-nitrite adducts. Color coding: iron, green; carbon, grey; nitrogen, blue; oxygen, red; hydrogen, white.

Fig. 5 – Crystal structure of the myoglobin-nitrite adduct, with the inset featuring a close-up view of the nitrite bound to the heme via the oxygen, not nitrogen atom.[48]



Our current research interests remain focused, among others, on exploring nitrite linkage isomerism, with the ultimate aim of providing insight into inorganic catalysis as well as microbiological and medical issues related to nitrite metabolism.

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REFERENCES

1. D. J. Richardson and N. J. Watmough, *Curr. Opin. Chem. Biol.*, **1999**, *3*, 207-219.
2. O. Einsle, A. Messerschmidt, R. Huber, P. M. H. Kroneck and F. Neese, *J. Am. Chem. Soc.*, **2002**, *124*, 11737-11745.
3. R. Silaghi-Dumitrescu, *Eur. J. Inorg. Chem.*, **2003**, 1048-1052.
4. R. Silaghi-Dumitrescu, *Inorg. Chem.*, **2004**, *43*, 3715-3718.
5. R. Silaghi-Dumitrescu, *J. Inorg. Biochem.*, **2006**, *100*, 396-402.
6. R. Silaghi-Dumitrescu, E. D. Coulter, A. Das, L. G. Ljungdahl, G. N. Jameson, B. H. Huynh and D. M. J. Kurtz, *Biochemistry*, **2003**, *42*, 2806-2815.
7. R. Silaghi-Dumitrescu, K. Y. Ng, R. Viswanathan and D. M. Kurtz, Jr., *Biochemistry*, **2005**, *44*, 3572-9.
8. M. T. Gladwin, *Nat. Chem. Biol.*, **2005**, *1*, 245-6.
9. K. Nakahata, T. Tanimoto, K. Hatano, K. Usuda and H. Shoun, *J. Biol. Chem.*, **1993**, *268*, 8350-8355.
10. S. Y. Park, *Nature struct. biol.*, **1997**, *4*, 827-832.
11. J. H. Enemark and R. D. Feltham, *Coord. Chem. Rev.*, **1974**, *13*, 339.
12. Y. Shiro, M. Fujii, T. Iizuka, S.-i. Adachi, K. Tsukamoto, K. Nakahara and H. Shoun, *J. Biol. Chem.*, **1995**, *270*, 1617-1623.
13. D. L. Harris, *Int. J. Quantum Chem.*, **2002**, *88*, 133-200.
14. T. S. Obayashi E and Shiro Y, *J. Am. Chem. Soc.*, **1998**, *120*, 12964-12965.
15. A. Daiber, T. Nauser, N. Takaya, T. Kudo, P. Weber, C. Hultschig, H. Shoun and V. Ullrich, *J. Inorg. Biochem.*, **2002**, *88*, 343-352.
16. B. A. Averill, *Chem. Rev.*, **1996**, *96*, 2951-2964.
17. M. Sono, M. P. Roach, E. D. Coulter and J. H. Dawson, *Chem. Rev.*, **1996**, *96*, 2841-2888.
18. R. Silaghi-Dumitrescu, *Studia Univ. Babes-Bolyai, Chemia*, **2006**, *51*, 167-174.
19. R. Silaghi-Dumitrescu,I. Silaghi-Dumitrescu, *Rev. Roum. Chim.*, **2004**, *3-4*, 257-268.
20. R. Silaghi-Dumitrescu, *Rev. Chim.(Bucharest)*, **2004**, *55*, 496-498.
21. R. Silaghi-Dumitrescu, B. J. Reeder, P. Nicholls, C. E. Cooper and M. T. Wilson, *Biochem. J.*, **2007**, *403*, 391-5.
22. R. Silaghi-Dumitrescu,C. E. Cooper, *Dalton Trans.*, **2005**, 3477-3482.
23. R. Silaghi-Dumitrescu, *J. Biol. Inorg. Chem.*, **2004**, *9*, 471-476.
24. R. Silaghi-Dumitrescu, *Arch. Biochem. Biophys.*, **2004**, *424*, 137-140.
25. D. M. Kurtz Jr., *Essays in Biochemistry*, **1999**, 55-80.
26. R. Silaghi-Dumitrescu and I. Silaghi-Dumitrescu, *J. Inorg. Biochem.*, **2006**, *100*, 161-6.
27. K. P. Jensen, B. O. Roos and U. Ryde, *J. Inorg. Biochem.*, **2005**, *99*, 45–54, Erratum on page 978.
28. J. H. Rodriguez, Y. M. Xia and P. G. Debrunner, *J. Am. Chem.Soc.*, **1999**, *121*, 7846-7863.
29. C. A. Brown, M. A. Pavlovski, T. E. Westre, Y. Zhang, B. Hedman, K. O. Hodgson and E. I. Solomon, *J. Am. Chem. Soc.*, **1995**, *117*, 715-732.
30. C. Hauser, T. Glasser, E. Bill, T. Weyhermuller and K. Wieghardt, *J. Am. Chem. Soc.*, **2000**, *122*, 4352-4365.
31. R. Silaghi-Dumitrescu and D. M. Kurtz Jr., *Chemtracts Inorg. Chem.*, **2003**, *16*, 468-473.
32. Y. Zhang and E. Oldfield, *J. Am. Chem. Soc.*, **2004**, *126*, 9494-9495.
33. D. M. Kurtz Jr., R. Silaghi-Dumitrescu, A. Das, G. Jameson, L. Ljungdahl and B. H. Huynh, *J. Inorg. Biochem.*, **2003**, *96* .
34. R. Silaghi-Dumitrescu, D. M. Kurtz, Jr., L. G. Ljungdahl and W. N. Lanzilotta, *Biochemistry*, **2005**, *44*, 6492-501.
35. E. C. Wasinger, M. I. Davis, M. Y. M. Pau, A. M. Orville, J. M. Zaleski, B. Hedman, J. D. Lipscomb, K. O. Hodgson and E. I. Solomon, *Inorg. Chem.*, **2003**, *42*, 365-376.
36. A. Wanat, T. Schneppensieper, G. Stochel, R. van Eldik, E. Bill and K. Wieghardt, *Inorg. Chem.*, **2002**, *41*, 4-10.
37. Z. Zhang, J. Ren, K. Harlos, C. H. McKinnon, I. J. Clifton and C. J. Schofield, *FEBS Lett.*, **2002**, *517*, 7-12.
38. P. L. Roach, I. J. Clifton, C. M. Hensgens, N. Shibata, C. J. Schofield, J. Hajdu and J. E. Baldwin, *Nature*, **1997**, 827-830.

39. T.-C. Yang, M. Wolfe, M. B. Neibergall, Y. Mekmouche, J. D. Lipscomb and B. M. Hoffman, *J. Am. Chem. Soc.*, **2003**, *125*, 2034-2035.
40. M. D. Clay, C. A. Cosper, F. E. Jenney, M. W. Adams and M. K. Johnson, *Proc. Natl. Acad. Sci. USA*, **2003**, *100*, 3796-3801.
41. M. Momenteau and C. A. Reed, *Chem. Rev.*, **1994**, *94*, 659-698.
42. G. Schenk, M. Y. Pau and E. I. Solomon, *J. Am. Chem. Soc.*, **2004**, *126*, 505-515.
43. P. A. Williams, V. Fulop, E. F. Garman, N. F. W. Saunders, S. J. Ferguson and J. Hajdu, *Nature*, **1997**, *389*, 406-412.
44. C. D. Richter, J. W. A. Allen, C. W. Higham, A. Koppenhoffer, R. S. Zajicek, N. J. Watmough and S. J. Ferguson, *J. Biol. Chem.*, **2002**, *5*, 3093-3100.
45. E. I. Tocheva, F. I. Rosell, A. G. Mauk and M. E. Murphy, *Science*, **2004**, *304*, 867-70.
46. E. V. Kudrik, S. V. Makarov, A. Zahl and R. van Eldik, *Inorg. Chem.*, **2005**, *44*, 6470-5.
47. E. V. Kudrik, S. V. Makarov, A. Zahi and R. van Eldik, *Inorg. Chem.*, **2003**, *42*, 618-24.
48. D. M. Copeland, A. S. Soares, A. H. West and G. B. Richter-Addo, *J. Inorg. Biochem.*, **2006**, *100*, 1413-25.