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NITRITE LINKAGE ISOMERISM IN BIOINORGANIC CHEMISTRY – A CASE FOR MECHANISTIC PROMISCUITY

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ABSTRACT. Reduction of nitrite to nitric oxide is essential in certain living species, and has even been proposed to be an important secondary function of hemoglobin in humans. In vivo, nitrite reduction is accomplished by metalloenzymes, and involves direct metal-nitrite coordination at iron, copper, and possibly molybdenum. Using density functional (DFT) results, we have proposed that linkage (nitro/nitrito) isomerism is an essential part of the mechanism in one class of nitrite reductase enzymes. Here, DFT data is shown suggesting the generality of nitrite linkage isomerism in bioinorganic chemistry, and experimental data supporting this theory-driven proposal is briefly reviewed. The concept that nitrite reduction may be achieved by a given metalloprotein via two different mechanisms with the same product and comparable efficiencies is considered, as part of our recently-defined mechanistic promiscuity paradigm.

Keywords: *nitrite reductase, heme, hemoglobin, myoglobin, DFT, nitrate reductase, nitrite, nitric oxide*

INTRODUCTION

Under anaerobic conditions, many microorganisms can sustain growth by using nitrate as respiratory terminal electron acceptor [1]. Within these systems, 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). Nitrate reduction to nitrite is catalyzed by molybdopterin-containing nitrate reductases. 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) [2-4]. 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].

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In addition to the above-discussed 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. The mechanisms whereby nitrite is reduced by hemes thus begin to entail increasing medical relevance [5].

RESULTS AND DISCUSSION

We have recently proposed that linkage nitro/nitrito isomerism is an essential part of the mechanism in the cases of copper and of heme d_1-containing nitrite reductases (NIR) [4,6]. Nitrite reduction by cytochrome cd_1 nitrite reductase (cd1NIR) had long been proposed to occur via N-coordination of nitrite to the d_1 heme of cd1NIR. 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.

Our group has however employed density functional theory (DFT) calculations to explore an alternative possibility, involving linkage isomerism of the nitrite at the NIR site [4,7]. Although the N- isomer (nitro) was found to be 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.

A revised catalytic cycle for cd1NIR is thus illustrated in Scheme 2. This mechanism 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.[4,7]

Reduction of nitrite to nitric oxide is also catalyzed by copper-containing NIR (Cu-NIR).[6,8] The proposed catalytic mechanism, illustrated in Scheme 2, has recently been confirmed by our own computational investigations.

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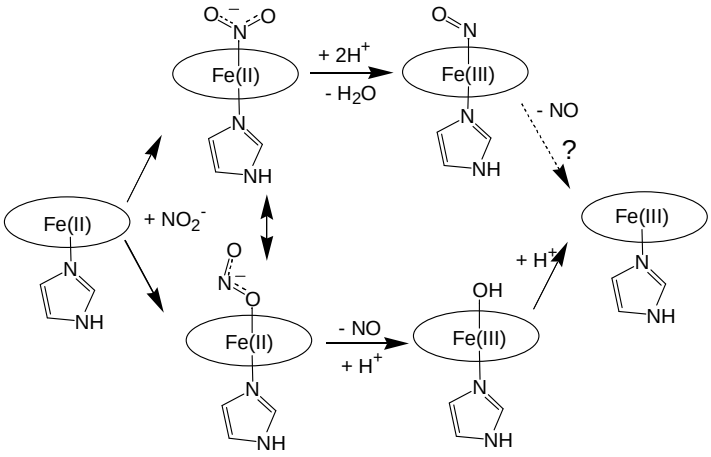
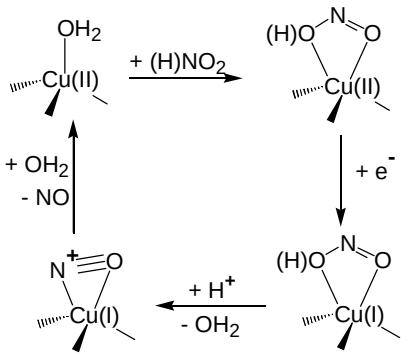


Scheme 1



Scheme 2

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 [9,10]. Thus, the reactions of nitrate and nitrite with sodium dithionite in the presence of various metallo-macrocycles in aqueous alkaline solution lead to different products (nitrous oxide and ammonia, respectively). These differences were explained in terms of different structures of the intermediate complex between CoI 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 [4,9].

Recent crystallographic work has for the first time established that nitrite coordinates to the iron in a hemoproteins, myoglobin, via the oxygen atom [11]. This result, supported by our unpublished EPR data, provides strong support for our linkage isomerism proposal in the case of cd1NIR.

Molybdenum-containing nitrate reductases from plants have more recently been proposed to be able to also reduce nitrite to nitric oxide [12]. Our unpublished DFT results support a role for linkage isomerism in this process.

CONCLUSIONS

Our current research interest is to explore nitrite linkage isomerism, attempting to establish trends and rules that govern this isomerism, with the ultimate aim of providing insight into inorganic catalysis as well as microbiological and medical issues related to nitrite metabolism. One of our working hypotheses is that nitrite reduction may be achieved by a given metalloprotein via two different mechanisms with the same product and comparable efficiencies; this would provide further support for our recently-defined mechanistic promiscuity paradigm, within the framework of which enzymes able to catalyze the same reaction via more than one mechanism may present an evolutionary advantage [13, 14].

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