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Forum

THE BASIC CHEMISTRY OF NITROGEN MONOXIDE AND PEROXYNITRITE

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Abstract—After a discussion of the physical chemistry of nitrogen monoxide, such as solubility (1.55 mM at 37°C and an ionic strength of 0.15 M) and diffusion constant (4.8×10^{-5} cm^2s^{-1}), several reactions that can acts as sinks are discussed, namely the reaction with dioxygen, with thiols and with superoxide. Of these, the latter reaction leads to a powerful oxidant, peroxynitrite. The thermodynamic and kinetic properties of this molecule are also reviewed. © 1998 Elsevier Science Inc.

Keywords—Nitrogen monoxide, Peroxynitrite, Kinetics, Thermodynamics, Reduction potential, Superoxide dismutase, Review

INTRODUCTION

The molecule nitrogen monoxide, known in biochemical and biomedical circles by its outdated[1,2] name "nitric oxide," serves as a messenger by binding to the heme of guanylate cyclase and, indirectly, as a cytotoxic agent. These discoveries and the formation of nitrogen monoxide from L-arginine, dating from the late 1980s,[3–7] opened a new field of research. Soon after the historical discovery of superoxide dismutase,[8] superoxide was considered a reactive, harmful radical. The same has happened to nitrogen monoxide. Although it is a radical, it is relatively stable, and like superoxide, it is not very reactive. The only documented harmful reactions of nitrogen monoxide are binding to the iron(II)hemes of cytochrome *c* oxidase[9,10] and hemoglobin,[11] and the conversion of oxyhemoglobin and oxymyoglobin to their "met" forms.[12–14] On the positive side, nitrogen monoxide reacts rapidly with organic radicals and stops radical chain reactions,[15,16] which is clearly beneficial in the short run. However, the accumulation of various organic nitrites and peroxynitrites may prove to be harmful over longer times.

Although the finding that nitrogen monoxide plays such an important role in biology is exciting, it is regrettable that a substantial amount of knowledge about nitrogen monoxide, which already existed before these discoveries were made, is being ignored. Some of these older findings will be discussed in this review in the context of new observations on the reactivity of nitrogen monoxide and its reaction products.

SOLUBILITY, LIFETIME, AND DIFFUSION

The concentration of nitrogen monoxide in a solution exposed to a partial pressure of 1 atm (101.3 kPa) of that gas is 1.93 mM at 25°C and 1.63 mM at 37°C (Table 1),[17] as calculated from data originally obtained by Winkler around the turn of the century. The temperature dependence is shown in Table 1. The solubility of a sparingly soluble gas like nitrogen monoxide is diminished by increasing ionic strength. No quantitative data appear to be available, and therefore it is assumed that the solubility of nitrogen monoxide is affected in the same way as that of dioxygen, namely a reduction by approximately 5% at 150 mM ionic strength.[18] This may be an overestimation, as nitrogen monoxide is more polar

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W. H. Koppenol was educated at the University of Utrecht, where he received a Ph.D. in radiation chemistry in 1978. Postdoctoral studies at Northwestern University centered around the electrostatic properties of cytochrome *c* and superoxide dismutase. In 1981, he joined the faculty of the University of Maryland Baltimore County and worked on superoxide dismutase mimics, the Fenton reaction, and thermodynamics of oxyradical reaction. The latter earned him the Iron Bolt, an award presented at the Oxyradical Gordon Conference of 1983. In 1987, he headed south to Louisiana State University in Baton Rouge, where he continued to study the mechanism of the Fenton reaction and started to work on peroxynitrite. Since 1994, he has been Professor of Bioinorganic Chemistry at the ETH in Zürich. From 1991 until 1997, he was a member of the IUPAC Committee for the Nomenclature of Inorganic Chemistry.

Table 1. Solubilities of Nitrogen Monoxide and Dioxygen in Water as a Function of Temperature

Temperature (°C)	$NO^{\bullet}$ (mM)[a]	O_2 (mM)[18b]
5	2.88	1.90
10	2.57	1.68
15	2.31	1.51
20	2.10	1.36
25	1.93	1.23
30	1.79	1.13
35	1.67	1.02
40	1.57	nc

Concentration of gas in water was in equilibrium with a partial pressure of 1 atm (101.3 kPa) at the indicated temperature.
[a] Calculated from thermodynamic tables.[17]
[b] Calculated from data valid for a partial pressure of 0.2091 atm by application of Henry's law.
nc: no data at a partial pressure of 0.2195 atm and at 40°C available.

Table 2. Reduction Potentials of Nitrogen and Oxygen Containing Species

Couple	E°′ (V)[a]
$NO^+/NO^{\bullet}$	1.21
$NO^{\bullet}/NO^-$(triplet)	0.39
$NO^{\bullet}/NO^-$(singlet)	−0.35
$NO_2^+/NO_2^{\bullet}$	1.6
$NO_2^{\bullet}/NO_2^-$	0.99
$N_2O_3/NO^{\bullet},NO_2^-$	0.8
$ONOO^{\bullet}/ONOO^-$	0.2
$ONOO^-/NO_2^{\bullet}$	1.6

Data were obtained from the literature.[25,26,47,48,83]
[a] The reduction potentials apply to 1 molal concentrations at pH 7.

than dioxygen. With this correction the solubility of nitrogen monoxide is 1.55 mM at physiological ionic strength and temperature. A partition coefficient of 6.5 has been reported for the system 1-octanol/water,[19] which suggests that the solubility of nitrogen monoxide in membranes may be six- to sevenfold higher as in the aqueous phase. Indeed, as suggested,[19] membranes could act as a reservoir of nitrogen monoxide.

To act as a messenger nitrogen monoxide needs to bind to guanylate cyclase, which it does at nanomolar concentrations. In contrast, macrophages produce local concentrations of nitrogen monoxide that are two or three orders of magnitude higher. At the low "messenger" concentrations the reaction with dioxygen to form nitrogen dioxide, a termolecular reaction, proceeds very slowly: one can easily calculate that the half-life of nitrogen monoxide at a concentration of 10 nM in the presence of 10 μM dioxygen is 10^6 s, and that it can diffuse 26 cm from its point of origin.[20] Due to its rapid reaction with (oxy)hemoglobin,[13] the biological lifetime of nitrogen monoxide is close to 5 s. Even during this time it can diffuse over several cell diameters, and thereby carry out its function as a messenger.[21] The diffusion coefficient of nitrogen monoxide in water is 2.07×10^{-5} cm^2s^{-1} at 20°C,[22] nearly identical to that of carbon monoxide as expected for molecules of similar size. From the unusual temperature dependence of this constant,[22] one interpolates a value of $4.8 \pm 0.1 \times 10^{-5}$ cm^2s^{-1} at 37°C. A value of 3.3×10^{-5} cm^2s^{-1} at 37°C has been deduced from experiments with microelectrodes where diffusion of nitrogen monoxide from endothelial cells into aortic muscle cells was observed,[19] and $3.8 \pm 0.3 \times 10^{-5}$ cm^2s^{-1} at 37°C has recently been reported in brain tissue. Membranes slow down the diffusion of nitrogen monoxide, but do not appear to be a significant barrier.[24]

THERMODYNAMICS

The Gibbs energies of formation of nitrogen monoxide and various other nitrogen–oxygen compounds have been compiled elsewhere.[25] From these data the reduction potentials listed in Table 2 have been calculated. From the values listed it is clear that in vivo it is not possible to form the nitrosyl cation, NO^+, as there are no oxidizing compounds available with high enough reduction potentials.[25] Even if it were formed it would react quickly with water to form nitrite [dioxonitrate(1-)]. It is possible that a one-electron reduction of nitrogen monoxide takes place, since $E°'(NO^{\bullet}/NO^-) = 0.39$ V (1 *m* $NO^{\bullet}$),[26] see Table 2.

$NO^{\bullet}$, NO^-, O_2, and H_2O_2

The chemistry of NO^- [oxonitrate(1-)] has been studied by pulse radiolysis. It reacts rapidly with another nitrogen monoxide to form dioxodinitrate(1-), $N_2O_2^{\bullet -}$,[27–29] even trioxotrinitrate(1-), $N_3O_3^{\bullet -}$, which have an absorption band at 480 nm.[30]

$$NO^{\bullet} + NO^- \Leftrightarrow N_2O_2^{\bullet -} \quad (1)$$
$$k_f = 1.7 \times 10^9\ M^{-1}s^{-1}$$
$$k_r = 6.6 \times 10^4\ s^{-1}$$

$$N_2O_2^{\bullet -} + NO^{\bullet} \rightarrow N_3O_3^- \quad (2)$$
$$k = 3.0 \times 10^6\ M^{-1}s^{-1}$$

$$N_3O_3^- \rightarrow N_2O + NO_2^- \quad (3)$$
$$k = 2.35 \times 10^2\ s^{-1}$$

Knowing the rates for the forward and the backward reaction (Reaction 1) allows one to calculate the Gibbs energy of formation, $\Delta_f G°$, of $N_2O_2^{\bullet -}$, which is 34 kcal/mol.

Oxonitrate(1-) can also react with dioxygen,[31] Reaction 4, to form peroxynitrite [oxoperoxonitrate(1-)]:

$$NO^- + O_2 \rightarrow ONOO^- \quad (4)$$

and in analogy to the reaction of nitrogen monoxide with superoxide (see below) and the reaction of nitrogen monoxide with oxonitrate(1-), Reaction 1, this reaction is expected to be fast. Surprisingly, it was recently reported that Reaction 4 is slow because the lowest state of oxonitrate(1-) appears to be the singlet state, which does not react rapidly with triplet dioxygen.[32] Nevertheless, Reaction 4 deserves attention as another potential source of oxoperoxonitrate(1-) in vivo.

One finds sometimes the following reaction in the literature:

$$2NO^- + 2H^+ \rightarrow N_2O + H_2O \quad (5)$$

While there are no thermodynamic objections to this reaction, its occurrence is kinetically most unlikely, as the concentration of oxonitrate(1-) will be much lower than those of either dioxygen or nitrogen monoxide, and the rate constants of the reaction of oxonitrate(1-) with these diatomics (k_1 and k_4) are likely to be higher than with itself (k_5). Thus, oxonitrate(1-) disappears through the reactions with nitrogen monoxide or dioxygen.

It has been stated[33] that nitrogen monoxide reacts with hydrogen peroxide to form oxoperoxonitrate(1-).[34] This is not correct: under anaerobic conditions, no reaction is observed. It has recently been demonstrated that oxygen is required, and that the nitrosating species is dinitrogen trioxide.[35] In general, nitrogen monoxide is not an oxidizing species.

NO• and thiol (RSH)

Nitrosothiols may act like nitrogen monoxide as the endothelium-derived relaxing factor, or function as compounds that store nitrogen monoxide. Nitrogen monoxide does not react directly with a thiol; an electron acceptor is required. The chemistry of nitrosothiol formation has been reviewed in 1985[36] and more recently in 1995.[37] The reduction potential of the couple RSNO, H^+/RSH, NO•, Reaction 10, is estimated with a cycle consisting of Reactions 6–9. Regarding Reaction 7, the bond energy of the RS-NO bond has been estimated at 29.6 kcal/mol (Dr. Plathe-Müller, unpublished). With a $T\Delta_{rxn}S$ correction of 9.5 kcal/mol[38] one arrives at the listed 20 kcal/mol. It is further assumed that the solvation energies of Reactions 6 and 8 cancel each other.

$$RSNO(aq) \rightarrow RSNO(g) \quad (6)$$

$$RSNO(g) \rightarrow RS^{\bullet}(g) + NO^{\bullet}(g)$$
$$\Delta_{rxn}G^\circ = +20 \text{ kcal/mol} \quad (7)$$

$$RS^{\bullet}(g) + NO^{\bullet}(g) \rightarrow RS^{\bullet}(aq) + NO^{\bullet}(aq) \quad (8)$$

$$RS^{\bullet}(aq) + e^- + H^+ \rightarrow RSH(aq)$$
$$E^\circ = 1.31 \text{ V (pH 0)} \quad (9)$$

$$RSNO(aq) + e^- + H^+ \rightarrow RSH(aq) + NO^{\bullet}(aq)$$
$$E^{\circ\prime} \approx 0.4 \text{ V at pH 0} \quad (10)$$

The value of 0.4 V at pH 0 of results in a reduction potential $E^{\circ\prime}$(RSNO, H^+/RSH, NO•) of approximately 0.0 V at pH 7, with RSNO, NO•, and RSH present at 1 molal concentrations. Such a low value allows a number of compounds to act as electron acceptors, including dioxygen, as has recently been found.[39] Although the formation of superoxide is energetically uphill, it disappears rapidly in subsequent reactions, and therefore one should consider the possibility that the reaction of nitrogen monoxide with a thiol in the presence of dioxygen results in oxoperoxonitrate(1-), as in Reactions 11 and 12.[39]

$$RSH + NO^{\bullet} + O_2 \rightarrow RSNO + O_2^{\bullet -} + H^+ \quad (11)$$

$$O_2^{\bullet -} + NO^{\bullet} \rightarrow ONOO^- \quad (12)$$

Often it is not described which molecule acts as an electron acceptor of the reaction of nitrogen monoxide with a thiol, as in Reaction 10; indeed one wonders whether it is realized that this reaction is a redox reaction.

The combination of nitrogen monoxide with oxoperoxonitrate(1-) could possibly lead to nitrosation of compounds such as thiols, as shown by Reactions 13–16:

	$\Delta_{rxn}G^\circ$ (kcal/mol)	
$NO^{\bullet} + ONOO^-$ (ONOOH) $\rightarrow NO_2^{\bullet} + NO_2^-$ (H^+)	−27	(13)
$NO^{\bullet} + NO_2^{\bullet} \rightarrow N_2O_3$	−4	(14)
$N_2O_3 + H_2O \rightarrow 2NO_2^- + 2H^+$	−13	(15)
$N_2O_3 + RSH \rightarrow RSNO + NO_2^- + H^+$	−18	(16)

whereby Reaction 16 is the sum of Reactions 17–19:

$$N_2O_3 + e^- \rightarrow NO^{\bullet} + NO_2^- \quad E^{\circ\prime} = +0.80 \text{ V} \quad (17)$$

RSH

$$\rightarrow RS^{\bullet} + H^{+} + e^{-} \qquad E^{\circ\prime} = -0.90\ V^{40} \quad (18)$$

$$RS^{\bullet} + NO^{\bullet} \rightarrow RSNO \qquad -20 \quad (19)$$

Although all Gibbs energies are favourable, the products of Reaction 13 have not yet been identified.

ONOO^{-}

Superoxide reacts quickly with nitrogen monoxide to form the oxoperoxonitrate(1-) anion, the rate has been reported as approximately 5×10^{9} $M^{-1}s^{-1}$.[41–43] Flash photolysis of stable oxoperoxonitrate(1-) solutions at pH 12 leads to dissociation into nitrogen monoxide and superoxide. The kinetics of the recombination reaction yield a rate constant of 1.9×10^{10} $M^{-1}s^{-1}$.[44] The reason that the rate constants obtained by others[41–43] are smaller may be due that the formation of superoxide and nitrogen monoxide was not instantaneous as in the flash photolysis experiment,[44] but involved a cascade of reactions that may have been rate limiting.

The chemistry of oxoperoxonitrate(1-) has been reviewed.[45,46] From thermodynamic considerations[47,48] one calculates that oxoperoxonitrate(1-) is an oxidizing species: E°($ONOO^{-}$, $2H^{+}$/ $NO_2^{\bullet}$, H_2O) is 1.6 V at pH 7, and that it is unstable with respect to disproportionation to nitrogen dioxide and the nitrosyldioxyl radical, $ONOO^{\bullet}$.[47] The isomerization to nitrate, which requires hydronation, has an activation energy of 18 kcal/mol. The pKa of ONOOH is 6.8 in 0.10 M phosphate buffer. This constant is affected by the composition of the medium.[44] For instance, at very low phosphate concentrations the pKa is 6.5.[44,49] At and above the pKa, and at combined $ONOO^{-}$ and ONOOH concentrations that exceed 0.1 mM, an adduct is formed between the anion and the hydronated form. This is the species that may decay to nitrite and dioxygen.[44] These products have been observed by Pfeiffer et al.[50] above neutral pH.

During the isomerization to nitrate, a reactive intermediate is formed that can nitrate and hydroxylate phenolic compounds such as tyrosine and oxidize dimethylsulfoxide.[51] These reactions are first-order in oxoperoxonitrate(1-) and zero-order in the compound that is being hydroxylated, nitrated, or oxidized: the formation of the reactive intermediate is rate-limiting. It has been suggested[52,53] that hydrogen oxoperoxonitrate undergoes homolyis to form nitrogen dioxide and the hydroxyl radical, but this reaction is not feasible for thermodynamic and kinetic reasons.[47,48] Experimentally, the isomerization reaction has a low activation volume of 1.7 ± 1.0 cm^3mol^{-1}, much less than the 10 cm^3mol^{-1} expected for homolysis.[44] How, then, does hydrogen oxoperoxonitrate acid act as a strongly oxidizing agent? Oxoperoxonitrate(1-) is present in solution in the *cis*-form[54] (see Fig. 1). While only a single bond is drawn between nitrogen and the first peroxide oxygen, quantum mechanical calculations show that the bond has considerable double bond character. The energy barrier between the *cis* and *trans* forms amounts to approximately 24 kcal/mol for the anion, and approximately 10 kcal/mol less for the hydronated form. The *cis*-form of the anion is 3–4 kcal/mol more stable than the *trans*-form.[55] Because the nitration and hydroxylation of phenolic compounds and the oxidation of dimethylsulfoxide take place with the same rate constant as the isomerization to nitrate, and do not depend on the concentration the phenolic compound, it was concluded that the same intermediate that leads to nitrate is involved. We proposed that this intermediate may be a bent form of *trans*-hydrogen oxoperoxonitrate.[47]

This simple model does not account for the observation that even at high concentrations of compounds that react with the reactive intermediate, there is still a considerable proportion of hydrogen oxoperoxonitrate that forms nitrate. It has therefore been suggested that there is another pathway that leads directly to nitrate.[56] Given the proposed rate, the enthalpy of the transition state of this proposed reaction must be near that of the intermediate, 18 kcal above the ground state. With ab initio methods, an intermediate with approximately that energy has been discovered.[57] It is a hydrogen-bonded structure that can be depicted as OH··ONO. However, the calculation applies to an intermediate in the gas phase and furthermore, this structure does not appear compatible with a kinetic deuterium isotope effect of 1.6 on the isomerization reaction.[84] The suggestion that isomerisation proceeds via a nitrogen dioxide and a hydroxyl radical in a cage cannot be experimentally verified, as radicals in a cage cannot be scavenged.[58] However, the hydroxyl radical is known to react with nitrogen dioxide to form hydrogen oxoperoxonitrate; nitrate has not been mentioned as a product.[59,60] In conclusion, the structure of the intermediate is not known, and the same applies to the transition state of the direct pathway.

Whether hydrogen oxoperoxonitrate undergoes homolysis or reacts via a *trans* intermediate, is irrelevant for biology. Any oxoperoxonitrate(1-) formed in blood vessels will react with carbon dioxide, and in cells with thiols. The second-order rate constants for these two reactions are 2×10^3 $M^{-1}s^1$ (see References 61 and 62) and 6×10^3 $M^{-1}s^1$,[63] respectively; multiplied with local concentrations of ~1 mM and ~5 mM, repectively, at 37° C and pH 7.4

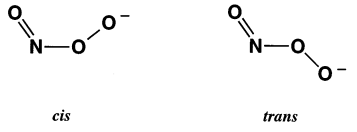

Fig. 1. The two conformations of oxoperoxonitrate(1-).

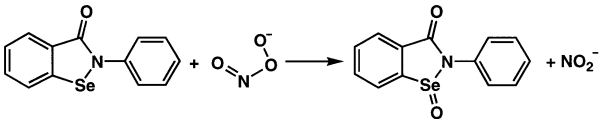

Fig. 2. The reaction of oxoperoxonitrate(1-) with ebselen.

one estimates rates of disappearence of approximately 2 s^{-1} and 30 s^{-1}, respectively, faster than the rate that leads to the oxidizing intermediate, which is 1 s^{-1} at physiological pH and 37°C.[48]

The nitration reaction is enhanced by some metal complexes, such as Fe(III)edta.[64,65] Surprisingly, it was found that Cu/Zn superoxide dismutase also catalyzes the nitration of a tyrosine analog.[64] This observation led to a hypothesis: In 25% of all cases of familial amyotrophic lateral sclerosis there is a mutation in the Cu/Zn superoxide dismutase.[65] More than 50 different mutations have been described so far. It has been suggested that any of these mutations makes the copper of superoxide dismutase better accessible to oxoperoxonitrate(1-), so that it becomes a better nitration catalyst.[66] Nitrated proteins[67] and free nitrotyrosine[68] have been found in affected nerve cells.

It would be advantageous if oxoperoxonitrate(1-) could be scavenged. This requires a compound that react with oxoperoxonitrate(1-) in a bimolecular fashion, and faster than carbon dioxide, which also forms an adduct with oxoperoxonitrate(1-) and enhances its nitrating capabilities.[61,69,70] Ascorbate reacts too slowly,[71] but it may be good at repairing damage.[72] Two compounds have been studied that would scavenge oxoperoxonitrate(1-) at micromolar concentrations, namely ebselen and an iron(III) porphyrin [5,10,15,20-tetrakis(*N*-methyl-4′-pyridyl)porphinatoiron(III)], which have rate constants of 2.0×10^6 $M^{-1}s^{-1}$ at 25°C[73] and 2.2×10^6 $M^{-1}s^{-1}$ at 37°C,[49] respectively. The ebselen oxide formed (Fig. 2) can possibly be regenerated in vivo by glutathione reductase, whereas the iron(III)porphyrin is a true catalyst.

Based simply on kinetics, and in the absence of catalysis by metal complexes, nitration of tyrosines in cells should be a late event. However, experiments show that tyrosine nitration takes place before the thiols are depleted (Prof. P. Nicotera, Konstanz, personal communication). There are two ways to explain this: (i) the cell is inhomogeneous and this simple "homogeneous solution" approach is not valid, or (ii) the carbon dioxide enhanced nitration proceeds faster than thiol oxidation, which would make it a physiologically significant process. The latter appears to be true.[74,75]

ROLE OF SUPEROXIDE DISMUTASE

Another approach taken by nature is to prevent the formation of oxoperoxonitrate(1-). Under normal conditions the amount of superoxide dismutase is sufficient to channel all superoxide towards the dismutation products dioxygen and hydrogen peroxide (Fig. 3). The product of the rate constant of superoxide with superoxide dismutase (2.4×10^9 $M^{-1}s^{-1}$)[76] with the superoxide dismutase concentration (approx. 10 μM) gives a rate of superoxide disappearance of 2×10^4 s^{-1}. Normal nitrogen monoxide concentrations are in the nanomolar range, say 10 nM. This concentration, multiplied with the rate constant of 2×10^{10} $M^{-1}s^{-1}$ for the reaction of nitrogen monoxide with superoxide, yields a rate of disappearance of 2×10^2 s^{-1}, much smaller than the 2×10^4 s^{-1} calculated above. Near activated macrophages the situation is different. With an estimated local nitrogen monoxide concentration of 10 μM the rate of oxoperoxonitrate(1-) formation is now 4×10^4 s^{-1}, which is twice the rate of superoxide disappearance through superoxide dismutase. We must conclude that superoxide dismutase cannot prevent the formation of oxoperoxonitrate(1-) near activated macrophages! For these calculations the superoxide dismutate concentration was assumed to be 10 μmol. Near activated macrophages, outside the cell, this concentration is likely to be lower. Therefore, oxoperoxonitrate(1-) formation is underestimated near these cells.

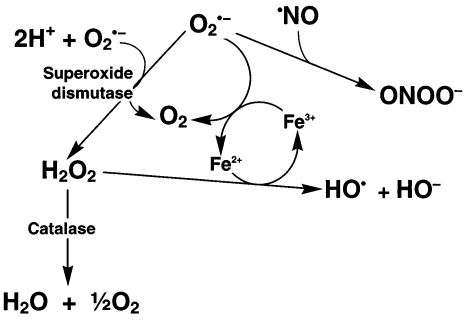

Fig. 3. Superoxide dismutase competes with nitrogen monoxide for superoxide. The left branch prepresents the classical view of hydroxyl radical-initiated damage. The right branch leads directly to an oxidizing species, oxoperoxonitrate(1-).

Figure 3 may also explain why formation of the hydroxyl radical via the one-electron reduction of hydrogen peroxide by Fe(II), the Fenton reaction,[77,78] is not likely kinetically: for the iron(III)edta complex the rate constant for the reduction of iron(III) by superoxide is of the order of 10^6 $M^{-1}s^{-1}$.[79] Although this rate constant is fast, it is still three orders of magnitude slower than the reaction of superoxide with superoxide dismutase.[76] In fact, there is little or no direct proof for the involvement of iron in vivo, except in cases of iron-related disorders. Most importantly, no iron complex has been identified in vivo that participates in the Fenton reaction. If the nature and the amount of these iron complexes are unknown, then it is not known how fast they are reduced by superoxide and oxidized by hydrogen peroxide. It has been shown that the rate constants are influenced by the coordination of the iron, because both

reactions proceed by inner-sphere electron transfer.[78] The role of the hydroxyl radical as the initiator of tissue damage has been questioned before.[20,80] Oxoperoxonitrate(1-) reactions produce compounds that have been taken as evidence for hydroxyl radical formation, such as salicylate hydroxylation,[65] initiation of lipid peroxidation,[81] and spin trap-OH formation.[82] Therefore, in systems where nitrogen monoxide is produced, one should first think about hydrogen oxoperoxonitrate as the initiator of oxradical damage: Exit $HO^{\bullet}$, intrat ONOOH.

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