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Reactivity of Reduced Nitroprusside, $[Fe(CN)_5NO^\bullet]^{3-}$, toward Oxygen

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The autoxidation reaction of nitrogen monoxide ($NO^\bullet$) is of fundamental concern, relevant to environmental chemistry and to modern aspects of NO biochemistry.[1] Studies in the gas phase and nonprotic media reveal that NO_2 is the main product, whereas in water, the reaction yields nitrite (eq 1).[2,3]

$$4NO^\bullet + O_2 + 2H_2O \rightarrow 4H^+ + 4NO_2^- \tag{1}$$

$NO^\bullet$ is synthesized in mammalian cells from L-arginine, involving the heme-containing enzyme NO synthase.[4] Reaction 1 might be partially responsible for the slow decay of free $NO^\bullet$ in aerated media.[1,3] Other main sinks for $NO^\bullet$ are the much faster reactions with oxyhemo- and oxymyoglobins, with soluble guanylyl cyclase and with superoxide.[4]

For assessing the mutual reactivity of $NO^\bullet$ and O_2 in biological fluids in the presence of metal ions, we must consider the relevant coordination chemistry. There are few reports on the reactions of transition metal $NO^\bullet$ complexes with aqueous O_2.[3] We present a detailed kinetic and mechanistic study of the autoxidation reaction of such a complex, namely, $[Fe(CN)_5NO^\bullet]^{3-}$ ($\mathbf{I}^{3-}$), the one-electron reduction product of the nitroprusside ion (NP), which is a "gold standard" NO donor drug with a number of clinical uses.[5] $\mathbf{I}^{3-}$ is a stable species, well characterized in aqueous solution,[6] with a formation constant, $K_{NO} = 1.6 \times 10^7\ M^{-1}$ (25 °C, $I = 0.1$ M),[7] and moderately inert with respect to $NO^\bullet$ dissociation (k_{-NO}, $1.6 \times 10^{-5}\ s^{-1}$).[7] Its spin density is essentially confined to the nitrosyl moiety (ca. 65%).[8] An understanding of the autoxidation reactivity of $\mathbf{I}^{3-}$ is important for completing the picture of possible reactions of NP in bodily fluids. We also compare the results for $\mathbf{I}^{3-}$ with available results for other complexes, looking for a generalized mechanistic description of the autoxidation reactions.[3]

Figure 1 shows the exponential decay of $\mathbf{I}^{3-}$ in the presence of an excess of dissolved O_2. The titration experiment (Supporting Information 1) and the quantitative formation of NP establish the following stoichiometry (eq 2):

$$4\mathbf{I}^{3-} + O_2 + 2H_2O \rightarrow 4[Fe(CN)_5NO]^{2-} + 4OH^- \tag{2}$$

The pseudo-first-order rate constant, k_{obs}, correlates linearly with $[O_2]$, leading to a second-order rate law: $-1/4d[\mathbf{I}^{3-}]/dt = k_2[\mathbf{I}^{3-}][O_2]$, with $k_2 = (3.5 \pm 0.2) \times 10^5\ M^{-1}\ s^{-1}$ at 25 °C. Activation parameters are $\Delta H^\ddagger = 40 \pm 5\ kJ\ mol^{-1}$, $\Delta S^\ddagger = 12 \pm 16\ J\ K^{-1}\ mol^{-1}$ (range 15−35 °C). An excess of free CN^- had to be used to minimize *trans*-labilization of this ligand;[6a,9] otherwise, the oxidation rate *decreased* markedly. The rate constant was insensitive to changes in pH (9−11) and ionic strength (0.1−1 M). No H_2O_2 nor nitrate was found in the reaction medium. Moreover, $\mathbf{I}^{3-}$ proved to be unreactive toward peroxide. An independent experiment employing an excess of $\mathbf{I}^{3-}$ also leads to a pseudo-first-order reaction.

These results discourage two plausible mechanistic alternatives: (a) an endergonic outer-sphere electron-transfer reaction yielding superoxide ($E°_{O_2/O_2^-} = -0.33$ V,[10] $E°_{FeNO^+/FeNO} = -0.09$ V,[11] vs NHE), with ensuing formation of reactive peroxide; (b) oxygen coordination and reactivity at a site created by dissociation of $NO^\bullet$, an unlikely sequence given the low value of k_{-NO}.[7,12] Other $[Fe^{II}(CN)_5L]^{n-}$ ions ($L = CN^-$, py, NH_3, etc.) and even $[Fe^{II}(CN)_5H_2O]^{3-}$ holding a labile site ($E°_{Fe^{III}/Fe^{II}} \geq 0.40$ V) are unreactive toward O_2. In the latter case, *slow* metal-centered oxidation is only achieved in the presence of Fe^{II}(aq) providing a full $4e^-$ O_2 reduction via some association between the two metal centers and O_2.[13]

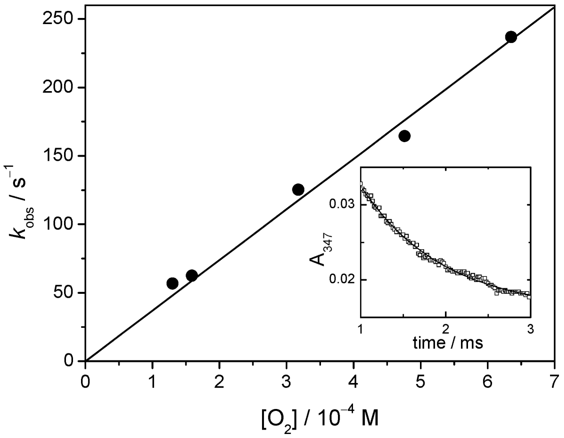

Figure 1. Plot of k_{obs} versus $[O_2]$ at 25.0 ± 0.1 °C; $\mathbf{I}^{3-} \approx 10^{-4}$ M; pH 10 (10^{-2} M Borax buffer), $[CN^-] = 5 \times 10^{-4}$ M; $I = 0.1$ M (NaCl). Inset: Stopped-flow trace for the reaction of a 10^{-4} M solution of $\mathbf{I}^{3-}$ with 0.26 mM $[O_2]$.

An attractive possibility involves association between bound $NO^\bullet$ and O_2. As a comparison, the rate law for reaction 1 was found to be $-1/4d[NO^\bullet]/dt = k_1[NO^\bullet]^2[O_2]$, with $k_1 = 2.88 \times 10^6\ M^{-2}\ s^{-1}$.[2] In the proposed mechanism, a weakly bound $NO^\bullet \cdots O_2$ complex is formed in the initial step, followed by the rate-determining reaction between this adduct and $NO^\bullet$, which leads to the intermediates ONOONO and $NO_2^\bullet$, precursors of the final nitrite formation. We propose eqs 3−4 as the initial mechanistic steps associated with reaction 2.

$$\mathbf{I}^{3-} + O_2 \underset{k_{-ad}}{\overset{k_{ad}}{\rightleftharpoons}} [Fe(CN)_5N(O)O_2]^{3-} \tag{3}$$

$$[Fe(CN)_5N(O)O_2]^{3-} + \mathbf{I}^{3-} \rightarrow 2[Fe(CN)_5NO_2^\bullet]^{3-} \tag{4}$$

Reaction 3 involves the formation of a new covalent bond between bound $NO^\bullet$ and O_2. According to our DFT computations (Supporting Information 2), the product can be best described as a peroxynitrite anion bound to Fe(III).[14] $[Fe(CN)_5NO_2^\bullet]^{3-}$ may further react as in eq 5. Both reactions 4 and 5 probably involve several steps, in which the oxidation equivalents remain bound to the metal,

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leading to the 4:1 global stoichiometry without other detectable byproducts:[15]

$$[\mathrm{Fe(CN)_5NO_2^{\bullet}}]^{3-} + \mathbf{I}^{3-} + \mathrm{H_2O} \rightarrow 2[\mathrm{Fe(CN)_5NO}]^{2-} + 2\mathrm{OH}^- \quad (5)$$

Assuming steady-state conditions for $[\mathrm{Fe(CN)_5N(O)O_2}]^{3-}$, we get $-\mathrm{d}[\mathbf{I}^{3-}]/\mathrm{d}t = 4k_{\mathrm{ad}}k_4[\mathrm{O_2}][\mathbf{I}^{3-}]^2/(k_{-\mathrm{ad}} + k_4[\mathbf{I}^{3-}])$. Under a limiting approach such that $k_4[\mathbf{I}^{3-}] > k_{-\mathrm{ad}}$, a first-order rate law in each reactant is obtained, with $k_2 = k_{\mathrm{ad}}$.

The use of metal nitrosyls to activate O_2 has long been of interest. Six-coordinated, formally $\mathrm{Co^{III}}$ and $\mathrm{Ir^{III}}$ angular *nitroxyl* complexes have been studied.[3] An initial electrophilic attack of O_2 on bound $\mathrm{NO^-}$ leads to N-bound peroxynitrite intermediates, which react in a competitive way to give nitrite, nitrate, or mixtures thereof as final products.[3]

Recent work shows that the spin density distribution along the $\{\mathrm{MNO}\}^7$ moiety remains essentially invariable in a series of $[\mathrm{ML_5NO^{\bullet}}]^n$ complexes (M = Fe, Ru) with different L-binding capabilities.[16] It is therefore reasonable to expect similar reactivity patterns for the $\mathrm{NO^{\bullet}}$ complexes. A second-order rate law has been found for the fast addition of O_2 on the $[\mathrm{Ru(NH_3)_5NO^{\bullet}}]^{2+}$ ion, with $k_{\mathrm{ad}} = 1.9 \times 10^6\ \mathrm{M^{-1}\ s^{-1}}$.[17] This was also the case for the $[\mathrm{Ru(bpy)(tpm)NO^{\bullet}}]^{2+}$ complex (bpy = 2,2′-bipyridine, tpm = tris(1-pyrazolyl)methane), although with a value of k_{ad} that is 5 orders of magnitude lower.[18] Interestingly, $\mathbf{I}^{3-}$ and $[\mathrm{Ru(NH_3)_5NO^{\bullet}}]^{2+}$ have very similar values of $E°_{\mathrm{MNO^+/MNO}}$ (ca. −0.1 V), while the value for $[\mathrm{Ru(bpy)(tpm)NO^{\bullet}}]^{2+}$ is 0.55 V.[17,18]

The faster autoxidation rates for the more reducing $\mathrm{NO^{\bullet}}$ complexes seem intuitively reasonable. More significantly, a tentative plot of ln k_{ad} against $E_{\mathrm{NO^+/NO}}$ for the three above-mentioned complexes shows a linear behavior, with a negative slope of $18.9 \pm 1.5\ \mathrm{V^{-1}}$ (Figure S3). Even though this linear free energy relation (LFER) should be tested with new measurements on appropriately designed $\mathrm{NO^{\bullet}}$ compounds, the result is encouraging. The value of the slope agrees with theoretical predictions based on the Marcus model for outer-sphere electron-transfer reactions, which has been extended to associative processes with inner-sphere character as those described above.[19] Remarkably, a similar plot with a *positive slope* has been found for the *electrophilic* addition reactions of $\mathrm{ML_5(NO^+)}$ complexes with $\mathrm{OH^-}$ as a nucleophile.[20]

Six-coordination appears to be a necessary condition to achieve oxidation of bound $\mathrm{NO^{\bullet}}$ and $\mathrm{NO^-}$ complexes. We discussed above the rate decrease for reaction 2 in the absence of external $\mathrm{CN^-}$, suggesting the *unreactivity* of $[\mathrm{Fe(CN)_4NO}]^{2-}$. These requirements have been also detected for an iron(II) nitrosyl porphyrin and for a non-heme complex in nonaqueous media.[21] In both cases, the two-electron processes occur with a 2:1 $\mathrm{Fe/O_2}$ stoichiometry and formally involve a one-electron oxidation of $\mathrm{NO^{\bullet}}$ to nitrite and a conversion of Fe(II) to Fe(III). The slow decay of moderately labile iron(II) nitrosyl porphyrins and hemoproteins $[\mathrm{Fe(P)NO^{\bullet}}]$ in aqueous aerated media is ambiguously understood.[3,22,23] Besides, uncertainties exist for the (estimated) high redox potentials for $\mathrm{NO^+}$ reduction in the heme compounds,[24] in contrast with the values for the robust complexes reported here. This work demonstrates that a broad reactivity range is displayed by different classical $\mathrm{NO^{\bullet}}$ complexes. That the rates could be controlled by the redox potentials of the bound $\mathrm{NO^+/NO^{\bullet}}$ couples appears as a challenging suggestion. Work with other $\mathrm{NO^{\bullet}}$ complexes, including heme-containing compounds, is currently underway.

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Supporting Information Available: Oxygen titration experiment (SI 1), details on the DFT calculations (SI 2), LFER plot of ln k_{ad} vs $E°_{\mathrm{MNO^+/MNO}}$ (SI 3), and complete ref 14. This material is available free of charge via the Internet at http://pubs.acs.org.

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(23) The reaction of $\mathrm{MbNO^{\bullet}}$ with O_2 occurs in two successive steps. The first one is pseudo-first-order in $\mathrm{MbNO^{\bullet}}$ ($k_{\mathrm{obs}} = 2.46 \times 10^{-4}\ \mathrm{s^{-1}}$, 25 °C, with $\Delta H^{\ddagger} = 120.5 \pm 6.8\ \mathrm{kJ\ mol^{-1}}$ and $\Delta S^{\ddagger} = 24 \pm 29\ \mathrm{J\ mol^{-1}\ K^{-1}}$), and the rate depends linearly on the oxygen pressure at low pressures.[22a] Different mechanisms have been considered, involving rate-determining NO dissociation (the value of k_{obs} is close to $k_{-\mathrm{NO}}$) or a reversible ligand exchange between $\mathrm{NO/O_2}$.[22a] A final irreversible electron transfer leads to metMb and nitrate. A similar dissociative mechanism has been proposed for $\mathrm{HbNO^{\bullet}} + \mathrm{O_2}$.[22b] Initial association of $\mathrm{MbNO^{\bullet}}$ with O_2 forming a N-bound peroxynitrite intermediate (as in eq 3) has also been postulated.[22c] Although negative values of $\Delta S^{\ddagger}$ are expected for associative processes, solvation effects must also be considered. Particularly, for reaction 3, cyano ligands bound to Fe(II) or Fe(III) interact very differently with water, favoring a more disordered situation for the Fe(III) product.[13]
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