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Reaction of Nitrogen Monoxide with a Macrocyclic Superoxorhodium(III) Complex Produces an Observable Nitratorhodium Intermediate

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Abstract: The rapid ($k \geq 10^6$ M^{-1} s^{-1}) reaction between NO and $L^2(H_2O)RhOO^{2+}$ (L^2 = *meso*-Me_6-[14]-ane-N_4) generates two strongly oxidizing, scavengeable intermediates, believed to be NO_2 and $L^2(H_2O)RhO^{2+}$. A mechanism is proposed whereby a peroxynitrito complex $L^2(H_2O)RhOONO^{2+}$ is formed first. The homolysis of O−O bond produces NO_2 and $L^2(H_2O)RhO^{2+}$ which were trapped with $ABTS^{2-}$ and Ni([14]aneN_4)$^{2+}$. In the absence of scavengers, the decomposition of $L^2(H_2O)RhOONO^{2+}$ produces both free NO_3^- and a rhodium nitrato complex $L^2(H_2O)RhONO_2^{2+}$, which releases NO_3^- in an inverse acid-dependent process. The total yield of $L^2(H_2O)RhONO_2^{2+}$ is 70%. In a minor, parallel path, NO and $L^2(H_2O)RhOO^{2+}$ react to give nitrite and the hydroperoxo complex $L^2(H_2O)RhOOH^{2+}$.

Introduction

Nitrogen monoxide, NO, reacts rapidly with both free and coordinated superoxide.[1−11] This chemistry is shown in eqs 1 and 2, where LMOO represents a superoxometal complex. Both the formation of peroxynitrous acid/peroxynitrite and its isomerization to nitrate in eq 1 are well documented.[1−4,6,8] It is now generally accepted that the isomerization involves homolytic O−O bond cleavage followed by the coupling of hydroxyl radicals with the nitrogen-centered NO_2 radicals.[1−4] About 30−40% of the radicals are scavengeable.

$$NO + O_2^{\bullet-}/HO_2^{\bullet} \rightarrow OONO^-/HOONO \rightarrow NO_3^- \quad (1)$$

$$NO + LMOO \rightarrow [LMOONO] \rightarrow \{NO_3^- + NO_2^-\} + LM \quad (2)$$

Much less is known about the intermediates in reaction 2, although a metal peroxynitrito species are probably involved. In support of such a scheme, a transient, believed to be a peroxynitritoiron complex, has been observed in the reaction of NO with oxyhemoglobin.[7] Also, a solid peroxynitrito complex of pentacyanocobalt(III), $(Et_4N)_3Co(CN)_5OONO$ has been prepared recently from $(Et_4N)_3Co(CN)_5OO$ and NO.[5]

The conversion of the coordinated peroxynitrite to free nitrate in the second step of eq 2 is commonly written[12] as in Scheme 1, in analogy with the isomerization of HOONO. According to this scheme, the homolytic cleavage of the peroxo bond (or heterolytic cleavage coupled with electron transfer)[12] is followed by isomerization within or outside of the solvent cage and dimerization and disproportionation of NO_2.[12−17]

The potential involvement of peroxynitrite in a number of pathological processes,[9,11−15] including cancer, heart disease, autoimmune diseases, and chronic inflammatory processes, has triggered a substantial effort to develop an understanding of catalytic isomerization of peroxynitrite and to examine the efficacy of metal complexes, mostly porphyrin-based, as isomerization catalysts. In most of the cases, the isomerization[12] is a complex process that involves a number of intermediates and metal oxidation states. Some compounds only accelerate the $OONO^- \rightarrow NO_3^-$ conversion,[13,14,16] while still involving free NO_2, a potent oxidizing and nitrating agent for biological tissues. Other compounds, alone or in combination with

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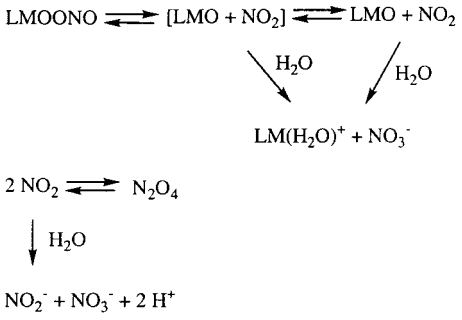
Scheme 1



biological reductants,[9,13,14,17,18] transform $OONO^-$ to NO_3^- by a route that mostly or completely bypasses NO_2, as judged by the protection that such catalysts provide against oxidation and nitration of biological tissues.[9,13,17,18]

The possibility that an intermediate metal nitrato complex is involved as a precursor to free nitrate has received an occasional brief mention in the literature,[9,12,19] but there has been no direct observation of such a species. With porphyrin complexes, the small spectral differences between the various intensely absorbing species may make such an intermediate difficult to detect. Also, the nitrate ion will dissociate rapidly from the iron and manganese centers typically used in such studies.[9,12−14,16,18]

We present here a study of the reaction between NO and a macrocyclic superoxorhodium complex, *β-trans*-[*meso*-(Me_6-[14]aneN_4)](H_2O)$RhOO^{2+}$, hereafter L^2RhOO^{2+}. We chose this particular complex in an attempt to stabilize potential intermediates, such as peroxynitrito and nitrato complexes. The substitutional inertness of Rh(III) was expected to slow hydrolytic processes at rhodium, although the choice of the metal would not have nearly as large or obvious control over homolytic O−O bond cleavage which is also expected to play a role in the decay of $L^2RhOONO^{2+}$.

Experimental Section

Materials. Perchloric acid, sodium perchlorate, sodium nitrate, sodium hydroxide, sodium sulfate, zinc metal, phosphoric acid, sulfuric acid, ammonium chloride, dimethylformamide (DMF), mercury(II) chloride (all Fisher), sodium nitrite, diphenylamine, antipyrine, p-aminobenzoic acid, 1-naphthol, 4,4'-sulfonyldianiline, hexaammineruthenium(III) chloride, diammonium salt of 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{2-}$), AscariteII (all Aldrich), and Sephadex SP−C25 were reagent grade or better and were used as received. $L^1Ni(ClO_4)_2$ (L^1 = *trans*-([14]aneN_4) was prepared as described previously.[20] Solutions of Fe_{aq}^{2+} and $Ru(NH_3)_6^{2+}$ were prepared by Zn amalgam reduction of the respective 3+ ions. Water was purified by passing the in-house distilled and deionized water through a Millipore-Q system.

Oxygen saturated solutions of L^2RhOO^{2+} were prepared as described previously[21] and were standardized spectrophotometrically, $\epsilon_{271} = 1.0 \times 10^4\ M^{-1}\ cm^{-1}$ (pH = 1−3) and $\epsilon_{265} = 1.12 \times 10^4\ M^{-1}\ cm^{-1}$ (pH = 11−12). For work under anaerobic conditions, the acidic solutions of L^2RhOO^{2+} were degassed with a strong stream of argon for 20 min. During this time, ∼40% of L^2RhOO^{2+} decomposed. Occasionally, an acidic solution of L^2RhOO^{2+} was first made alkaline by the addition of dilute NaOH, degassed for 40 min, and then reacidified. The loss of L^2RhOO^{2+} in such experiments was <10% owing to the much lower rate of homolysis of L^2RhOO^{2+} at alkaline pH. The two methods yielded identical kinetic and product data.

L^2RhOOH^{2+} was prepared by reduction of L^2RhOO^{2+} with a stoichiometric amount of $Ru(NH_3)_6^{2+}$ under argon. At pH = 3.3, the solutions of L^2RhOOH^{2+} showed <5% decomposition in 5 h.

NO gas (Matheson) was purified by passage through an ascarite column, two washing towers filled with 4 M NaOH and one washing tower filled with distilled water. Stock solutions of NO were prepared by bubbling the purified gas for 25 min through 20 mL of an argon-purged aqueous solution. When kept under excess NO, such solutions showed no significant deterioration in several hours. The concentrations of NO and NO_2^- were checked by determining the nitrite concentrations before and after the oxidation by O_2. A typical stock solution contained 1.7 mM NO and 0.2 mM nitrite ions.

Solutions of L^2RhOO^{2+} for the kinetics, stoichiometry, and product analysis were placed in a spectrophotometric cell and were degassed. The reaction was initiated by injecting a small volume (≥100 μL) of NO stock solution.

Kinetics measurements used a Shimadzu UV-3101PC spectrophotometer and an Applied Photophysics DX-17MV stopped-flow apparatus. Most of the kinetic studies were conducted at 25 ± 0.5 °C and 0.10 M ionic strength, maintained with perchloric acid and sodium perchlorate. Nonlinear least-squares fittings were carried out with the use of Kaleidagraph 3.0 for PC software.

Product Analysis. Nitrite concentrations were determined by either direct spectrophotometry at pH 1 ($\epsilon_{371} = 52.6\ M^{-1}\ cm^{-1}$, $pK_a = 3.2$),[22] or a standard nitrite test based on the reaction with 1-naphthol and *para*-aminobenzoic acid.[23] Molecular oxygen, NO_3^-, L^2RhOO^{2+}, and $L^2Rh(H_2O)_2^{3+}$ did not interfere. The molar absorptivity of the product in the 3−35 μM range was determined to be $\epsilon_{519} = 3.22 \times 10^4\ M^{-1}\ cm^{-1}$.

Prior to the quantification of free nitrate produced in the L^2RhOO^{2+}/NO reaction, the sample was passed through a short, ice-cold column of Sephadex C-25 to remove metal ions. The ion-exchange step was introduced to distinguish between free and bound nitrate. In control experiments with authentic nitrate and nitrite samples, the nitrite was completely absorbed by the column, and nitrate could be washed down quantitatively with 0.01 M $HClO_4$.

The sample solution was then neutralized with a dilute solution of NaOH, the volume was reduced by rotary evaporation, and nitrate analysis was carried out by one or both of the following methods. The first method is based on the reaction with antipyrine as described in the literature.[24] In our hands the precision was poor, and only the sum of nitrate and nitrite concentrations could be determined. The other method is a modification of a literature procedure employing 4,4'-sulfonyldianiline.[25] The test solution (1 mL) was mixed with 5 mL of the nitrate reagent (1 g of 4,4'-sulfonyldianiline, 1 g of NH_4Cl, 0.05 g of Ph_2NH in 1 L of 1:1 concentrated H_2SO_4 and H_3PO_4), and the absorbance increase, followed by a decrease, was monitored in the 600−800 nm range. The maximum absorbance at λ 600 nm was then used to construct a calibration plot for nitrate concentrations in the 10−100 μM range. The calibration curve was nonlinear and could be fitted to an approximate equation $Abs = 7.24 \times 10^5\ [NO_3^-]^{1.47}$. All the quantitative nitrate analyses reported later were obtained by this method.

To test for free peroxynitrite in spent reaction solutions, L^2RhOO^{2+} was allowed to react with NO at pH 12, where $OONO^-$ is stable, and the UV−vis spectrum was recorded. The reaction mixture was then acidified with $HClO_4$ to pH 2 to allow the peroxynitrite to isomerize to nitrate. The pH of the solution was again raised to 12, and the

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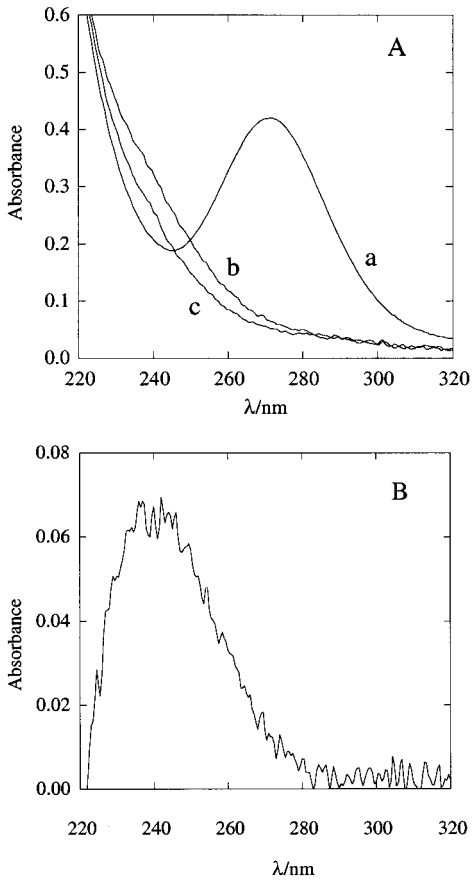

Figure 1. (A) Spectral changes observed upon mixing 42 μM L^2RhOO^{2+} (spectrum *a*) with 100 μM NO in 0.015 M $HClO_4$. Spectrum *b* was recorded immediately after the addition of NO to L^2RhOO^{2+} and spectrum *c* 200 min later. (B) Difference spectrum of the intermediate $L^2RhONO_2^{2+}$ (*b*−*c*).

spectrum was recorded. The two spectra were examined for differences at 302 nm where $OONO^-$ exhibits a maximum, $\epsilon = 1.70 \times 10^3\ M^{-1}\ cm^{-1}$.[26]

Results

General Observations. The reaction between L^2RhOO^{2+} and NO at pH 1−12 under anaerobic or aerobic conditions is instantaneous even on the stopped-flow time scale, which allowed us to place a lower limit for the rate constant at $\geq 10^6\ M^{-1}\ s^{-1}$.

Most of the experiments were carried out by use of conventional spectrophotometry. Upon mixing of the two reactants, the absorption maximum of L^2RhOO^{2+} at 271 nm disappeared and an intermediate was formed and then disappeared in a pH-dependent reaction with a maximum absorbance change around 240 nm at pH 1, as shown in Figure 1. Molecular oxygen had no effect on the outcome of the reaction, provided sufficient amount of NO was employed to make up for the losses caused by its autoxidation.

The stoichiometry was estimated in an experiment using excess L^2RhOO^{2+} (20−35 μM) over NO. In three independent runs, an approximate 1:1 stoichiometry was obtained. A more precise method utilized a spectrophotometric titration at 271 nm. The stoichiometry varied somewhat with the amount of oxygen left in the solution after degassing. In acidic solutions after 20 min of purging with argon, the stoichiometry was $[L^2RhOO^{2+}]/[NO] = 1{:}1.2$. At pH 11 after 40 min of degassing the ratio was 1:1.0, and in oxygen-saturated solutions the ratio was 1:1.6. In all the cases the same intermediate was produced, and deviations from the 1:1 stoichiometry appeared to be caused solely by the additional consumption of NO by autoxidation because of the imperfect (manual) mixing of the reagents and high local concentrations of NO. Even though the reaction of NO with L^2RhOO^{2+} is much faster than with O_2, the autoxidation is still fast at the point of injection where the concentration of NO is high (>1 mM).

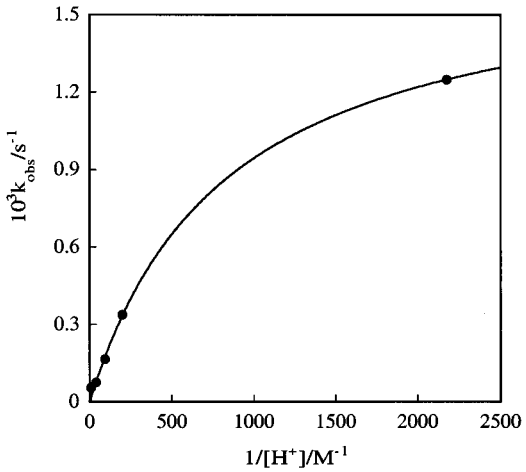

Figure 2. Plot of the rate constant for the decay of $L^2RhONO_2^{2+}$ against the inverse acid concentration according to eq 3.

Decay of the Intermediate. In acidic solutions, the absorbance decrease at 240 nm obeyed first-order kinetics. Figure 2 shows a plot of the observed first-order rate constants against $[H^+]^{-1}$ and the fit to eq 3, which yielded $k_h = (1.7 \pm 0.1) \times 10^{-3}\ s^{-1}$ and K_a (the acidity constant for the coordinated H_2O) $= (1.2 \pm 0.1) \times 10^{-3}$ M. The kinetics were unaffected by the presence of ≤0.1 mM NO, 0.1 mM NO_2^-, or 1 mM O_2.

$$k_{obs} = k_h K_a/(K_a + [H^+]) \quad (3)$$

Under basic conditions, there was some tailing in the absorbance at longer times. We were not able to identify the species causing this small and slow absorbance decrease, but the exponential fit through the first four half-lives for the reaction of interest was good and yielded $k = 2 \times 10^{-3}\ s^{-1}$ at pH 12. This rate constant is close to the limiting value ($1.7 \times 10^{-3}\ s^{-1}$) obtained from the data at pH < 3.3, showing that only two forms of the intermediate, related by a K_a of 1.2×10^{-3} M, exist in the pH range 1−12.

Reaction solutions before and after the decomposition of the intermediate were examined for the presence of free $OONO^-$, as described in the Experimental Section. No $OONO^-$ was detected. After the intermediate had completely decomposed, the solution contained mostly nitrate and some nitrite. On the basis of the initial concentration of L^2RhOO^{2+}, the yields were ∼90% and ∼10%, respectively.

Nitrate analysis was also carried out immediately after the L^2RhOO^{2+}/NO reaction, that is, when most of the intermediate was still intact. At that point, the amount of NO_3^- was 20% of $[L^2RhOO^{2+}]_0$, Table 1.

In another experiment, the reaction between L^2RhOO^{2+} and NO was conducted at pH 11. The solution was then acidified

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Table 1. Yields of Free Nitrate at Various Times during the Decomposition of the Intermediate[a]

pH	time/min	$[NO_3^-]/[L^2RhOO^{2+}]$	% decay[b]
3.3	0	0.20	0
	60	0.89	98
11[c]	0	0.21	0
	75	0.59	57
	360	0.91	98

[a] Initial concentrations: $[L^2RhOO^{2+}] = (25-100)\ \mu M$, $[NO] = (50-100)\ \mu M$. [b] Calculated from eq 3. [c] The reaction between L^2RhOO^{2+} and NO was carried out at pH 11, but the decay kinetics and nitrate analysis were performed after acidification to pH 2, see text.

Table 2. Summary of Rate Constants Determined in This Work[a]

reaction	$k/M^{-1}\ s^{-1}$
$L^2RhOO^{2+} + NO$	$>10^6$
$ABTS^{2-} + HONO$	60 ± 1
$L^2RhOOH^{2+} + Fe_{aq}^{2+}$	28 ± 2
$HONO + Fe_{aq}^{2+}$	1.58 ± 0.03[b]
$(HO)L^2RhONO_2^+ + H_2O$	$1.7\ (\pm 0.1)\ 10^{-3}$[c]

[a] At 25.0 °C, $[H^+] = 0.01-0.10$ M, $\mu = 0.10$ M. [b] $[H^+] = 0.10$ M. [c] K_a for $L^2(H_2O)RhONO_2^{2+} = 1.2 \times 10^{-3}$ M, see text and eq 3.

to pH 2 to quench the hydrolysis of the intermediate, and nitrate analysis was carried out as above. The yields at zero time, one half-life for the decomposition of the intermediate, and after the decay was mostly complete are also shown in Table 1. Apart from the initial 20%, the growth in NO_3^- clearly parallels the decay of the intermediate. The combined results at pH 3.3 and pH 11 establish that the same species is produced in quantitative yields throughout the entire pH range examined.

Reactions with Reducing Agents. When $ABTS^{2-}$ (85−170 μM) was added to the solution immediately after completion of the L^2RhOO^{2+}/NO reaction, there was a slow absorbance increase at 645 nm, where ϵ_{max} for $ABTS^{\bullet-}$ is $1.35 \times 10^4\ M^{-1}\ cm^{-1}$.[27] This was traced to the reaction with nitrite which is always present in small amounts in solutions of NO. The rate constant for the HONO/$ABTS^{2-}$ reaction was determined independently, $k = 60 \pm 1\ M^{-1}\ s^{-1}$ at pH 1, Table 2. There was no reaction between the intermediate and $ABTS^{2-}$.

The reaction with Fe_{aq}^{2+} (0.17−0.67 mM) exhibited biphasic kinetics. The absorbance changes at 240 nm were much smaller than expected for quantitative generation of Fe_{aq}^{3+} ($\epsilon = 4.16 \times 10^3\ M^{-1}\ cm^{-1}$) by the intermediate. The faster kinetic stage had $k = 28 \pm 2\ M^{-1}\ s^{-1}$, which is identical to the independently measured rate constant for the $L^2RhOOH^{2+}/Fe_{aq}^{2+}$ reaction, Table 2. The absorbance change for this stage could account for only ~10% of the initial L^2RhOO^{2+}.

The slower stage was again traced to nitrite. The rate constant coincided with that determined independently for the HONO/Fe_{aq}^{2+} reaction, $1.58 \pm 0.03\ M^{-1}\ s^{-1}$ at 0.10 M H^+, close to the literature value,[28] $3.4\ M^{-1}\ s^{-1}$ at an ionic strength of 2.1 M. There was no evidence for a reaction between the intermediate and Fe_{aq}^{2+}.

The search for short-lived intermediates was conducted in both O_2-saturated and O_2-free solutions at pH 1−4. NO (0.1−0.4 mM) was used in excess over L^2RhOO^{2+} (10−25 μM) to make up for air oxidation of NO during solution manipulations and transfer in these stopped-flow experiments.

All the L^2RhOO^{2+} disappeared in mixing time (3−4 ms). No other absorbance changes were observed at $230 < \lambda < 390$ nm, aside from those associated with the reaction of excess NO with traces of O_2.

The next set of experiments utilized (separately) two reagents, L^1Ni^{2+} and $ABTS^{2-}$, that were known[29] or presumed to react with some of the potential short-lived intermediates such as $L^2RhOONO^{2+}$, NO_2, and L^2RhO^{2+}. Two types of mixing, regular and sequential, were used in these experiments.

When an acidic ($[H^+] = 0.05$ M) solution containing L^2RhOO^{2+} and L^1Ni^{2+} was mixed with an equal volume of a solution containing excess NO (0.85 mM after mixing) in 0.1 M Na_2SO_4,[30] the absorbance at 307 nm increased in the mixing time, signaling the formation of L^1Ni^{3+} ($\epsilon = 1.1 \times 10^4\ M^{-1}\ cm^{-1}$).[31] The amount of L^1Ni^{3+} increased somewhat with the concentration of L^1Ni^{2+} used but reached saturation at 2.5 mM L^1Ni^{2+}. At that point, the amount of L^1Ni^{3+} formed was ~40% of the initial concentration of L^2RhOO^{2+}.

Similar experiments were conducted with $ABTS^{2-}$. The reaction was again completed in mixing time. The maximum yield of $ABTS^{\bullet-}$ in 0.05 M $HClO_4$ was 71% of the initial L^2RhOO^{2+}. The results were qualitatively comparable at pH 11, but the yield of $ABTS^{\bullet-}$ was only 44%. As expected, the absolute amount of $ABTS^{\bullet-}$ produced was linearly related to the initial concentration of L^2RhOO^{2+} and showed saturation with $ABTS^{2-}$, Figure 3.

In experiments utilizing sequential mixing, L^2RhOO^{2+} was first mixed with NO. After a 15-ms delay, the product solution was mixed with the scavenger, L^1Ni^{2+} or $ABTS^{2-}$. No reaction was observed with either scavenger, showing that the oxidant responsible for the formation of $ABTS^{\bullet-}$ and L^1Ni^{3+} in the standard stopped-flow experiments has a lifetime of less than 15 ms.

Discussion

Nature of Intermediates. The difference spectrum and the decay kinetics, Figures 1 and 2, clearly show that the observed intermediate is not HOONO, which exhibits only weak absorbance around 300 nm and decomposes in less than a second in acidic aqueous solutions.[8] The failure of the intermediate to react with Fe_{aq}^{2+} and $ABTS^{2-}$ argues also against the coordinated peroxynitrite, $L^2RhOONO^{2+}$. We would expect Fe_{aq}^{2+} to reduce this complex rapidly given that the related, but presumably less reactive hydroperoxo species L^2RhOOH^{2+} has $k_{Fe} = 28\ M^{-1}\ s^{-1}$.

The decay of the intermediate to release NO_3^- clearly points to the nitrato complex $L^2RhONO_2^{2+}$ as the most likely candidate. This assignment is also consistent with the observed base-catalyzed decomposition, a feature exhibited by most rhodium complexes in aqueous solution.[32] According to this interpretation, the major pathway for hydrolysis utilizes the hydroxo species, $L^2(OH)RhONO_2^+$, in the range of acid concentrations studied. Our attempts to independently synthesize

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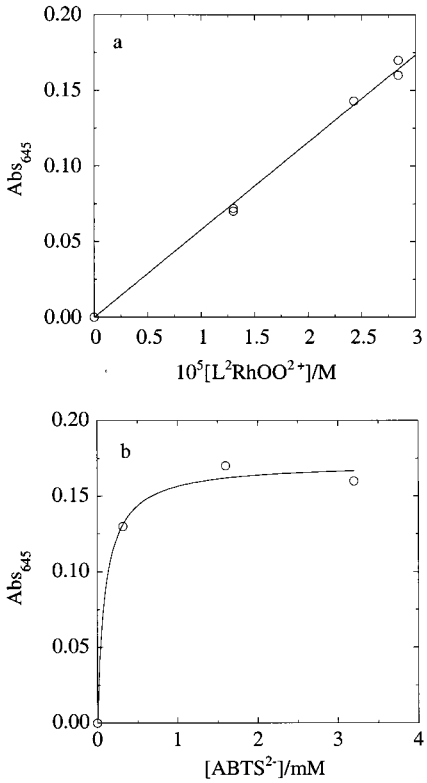


Figure 3. Plot of the absorbance at 645 nm immediately after mixing L^2RhOO^{2+}, $ABTS^{2-}$, and NO in a stopped flow as a function of (a) concentration of L^2RhOO^{2+} and (b) concentration of $ABTS^{2-}$. Conditions: argon atmosphere, $[NO]_0 = 0.85$ mM. In (a), $[ABTS^{2-}] = 1.3$ mM, pH 11.4. In (b), $[L^2RhOO^{2+}] = 28\ \mu$M, pH 11.1.

and characterize the nitratorhodium complex have so far been unsuccessful.

The value of the rate constant k_h is large[32] for a rhodium(III) complex, even for a weak ligand such as nitrate. The data suggest a mechanism involving cleavage of the nitrogen−oxygen bond of the nitrate rather than the rhodium−oxygen bond, in analogy to related reactions of oxoanion−transition metal complexes.[33−36] Interestingly, an anomalously large rate was also reported for another rhodium complex, $(NH_3)_5RhONO^{2+}$, in a related nitrito-to-nitro isomerization,[37] which features only partial cleavage of the rhodium−oxygen bond in the transition state.

The observations in the scavenging experiments strongly suggest that the two fragments, NO_2 and L^2RhO^{2+}, rather than their presumed precursor, $L^2RhOONO^{2+}$, are the active oxidants for L^1Ni^{2+} and $ABTS^{2-}$, eqs 4−6.

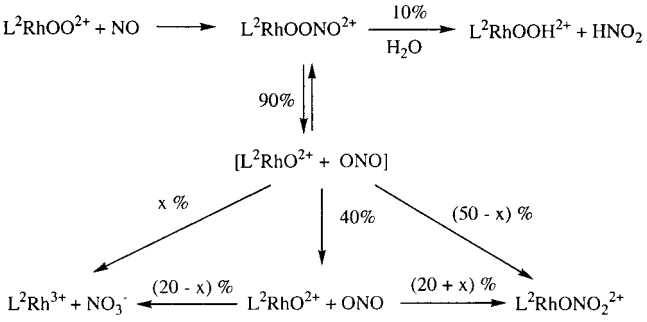
$$L^2RhOO^{2+} + NO \rightarrow [\,L^2RhOONO^{2+}] \rightarrow L^2RhO^{2+} + NO_2 \qquad (4)$$

$$L^2RhO^{2+} + ABTS^{2-} \xrightarrow{2\ H^+} L^2Rh^{3+} + ABTS^{\bullet-} + H_2O \qquad (5)$$

$$NO_2 \begin{cases} \xrightarrow{ABTS^{2-}} ABTS^{\bullet-} \\ \xrightarrow{L^1Ni^{2+}} L^1Ni^{3+} \end{cases} \qquad (6)$$

The approximate doubling of the yield of $ABTS^{\bullet-}$ relative to L^1Ni^{3+} requires both fragments to react with $ABTS^{2-}$, but only one to react with L^1Ni^{2+}. Bimolecular reactions of macrocyclic rhodium complexes with other metal macrocycles have been shown to be consistently slow.[21,38] It is thus reasonable that L^2RhO^{2+} would decompose by other routes, possibly by intramolecular electron transfer,[39,40] rather than to react with L^1Ni^{2+}. $ABTS^{2-}$, on the other hand, is much more reactive and apparently capable of scavenging L^2RhO^{2+}. The reactions of NO_2 with both $ABTS^{2-}$ ($k = 2.2 \times 10^7\ M^{-1}\ s^{-1}$)[41] and L^1Ni^{2+} ($k = 1.1 \times 10^8\ M^{-1}\ s^{-1}$)[29] are fast, accounting cleanly for our observations.

Scheme 2



A mechanism that takes into account all of our experimental observations is outlined in Scheme 2. All the percentages are expressed in terms of the initial concentrations of L^2RhOO^{2+}. The total yield of NO_3^- was 90%, of which 20% was produced "instantaneously" upon mixing of the reagents, Table 1, and 70% by the decay of $L^2RhONO_2^{2+}$. The two homolysis fragments, L^2RhO^{2+} and NO_2, undergo cage escape to the extent of 40%, as determined in the scavenging experiments with $ABTS^{2-}$ and L^1Ni^{2+}, and react to give the free and coordinated nitrate, just as the caged species do. The only unknown in the scheme is the fraction *x*, which determines the proportion of L^2RhO^{2+}/NO_2 reactions within and outside of the solvent cage.

The mechanism for the rapid generation of NO_3^-, that is, the fraction (20%) that bypasses $L^2RhONO_2^{2+}$, is not obvious. If an outer-sphere electron transfer is involved, then the reduction potential for the Rh(IV)/Rh(III) couple must be higher than 1.6 V, the value for the NO_2^+/NO_2 couple.[6]

As should be expected on the basis of the known chemistry of NO_2, the yield of $ABTS^{\bullet-}$ at pH 11 was significantly smaller (44%) compared to that obtained in acidic solutions (71%). Nitrogen monoxide, which was used in excess in these experiments, reacts rapidly ($k = 1.1 \times 10^9\ M^{-1}\ s^{-1}$)[42] with NO_2 as in eq 7. The reverse reaction is also fast, $k = 8 \times 10^4\ s^{-1}$,[42] which provides a pathway for NO_2 to be scavenged by $ABTS^{2-}$ in acidic solutions.

$$NO + NO_2 \rightleftharpoons N_2O_3 \qquad (7)$$

At pH 11, however, N_2O_3 hydrolyzes rapidly,[43] eq 8, such that the removal of NO_2 in eq 7 becomes in effect irreversible and

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the consumption of $ABTS^{2-}$ drops to a value that is only slightly greater than that required for the scavenging of L^2RhO^{2+} alone.

$$N_2O_3 + HO^- \rightarrow 2\,NO_2^- + H^+ \qquad k = 1 \times 10^8\ M^{-1}\ s^{-1}\ ^{43} \quad (8)$$

This argument is supported by the results of chemical simulations. The scheme consisting of reactions 4−8, and taking $k = 530\ s^{-1}$ for acid hydrolysis[42] of N_2O_3, predicts that a solution initially containing {30 μM L^2RhO^{2+} + 30 μM NO_2} will generate the following concentrations of $ABTS^{\bullet-}$: 60 μM (pH 1, [NO] = 20 μM), 57 μM (pH 1, [NO] = 0.8 mM), 55 μM (pH 11, [NO] = 20 μM), and 33 μM (pH 11, [NO] = 0.8 mM). The significant decrease in $[ABTS^{\bullet-}]$ is seen to occur only at high pH and high [NO], as experimentally observed. The observed decrease in the yield as the pH changes from 1 to 11 is 38%, in good agreement with the calculated 42%.

About 10% of the reaction yields L^2RhOOH^{2+} and HONO, both of which were observed experimentally. The heterolysis of O−O bond, Scheme 2, is the most likely mechanism for the formation of these minor products.

To our knowledge, this is the first instance where a nitratometal complex has been observed directly as an intermediate in an NO/superoxometal reaction. After this work was completed, we became aware of another nitrato complex, $(CN)_5CoONO_2^{3-}$,[44] which was produced by isomerization of $(CN)_5CoOONO^{3-}$.[5] The rhodium complexes in the present work would seem to be better models for the biological systems in that the isomerization is exceedingly fast (<3 ms). Both the peroxynitrito and nitrato complexes of pentacyanocobalt(III) are long-lived.[5,44]

Our results support the notion that metal−nitrato species are indeed involved in other metal-catalyzed isomerizations of peroxynitrite. In fact, the reactivity of LMO toward NO_2 (to generate $LMONO_2$ or $LM(H_2O)^+ + NO_3^-$, see Scheme 1) relative to the rate of cage collapse (to liberate NO_2) may play a role in determining the success of a particular catalyst in peroxynitrite isomerization. The more reactive the LMO, the greater the fraction of the fragments recombining within the solvent cage and the smaller the amount of the undesirable NO_2 diffusing freely into the solution. A rapid LMO/NO_2 reaction would also provide a second line of defense outside of the solvent cage by outcompeting the tissue-damaging nitration and oxidation. The rates of LMO/NO_2 reactions vary dramatically among iron porphyrin complexes.[9,14,19] Our simulations require a rate constant of $2 \times 10^8\ M^{-1}\ s^{-1}$ for the L^2RhO^{2+}/NO_2 reaction to explain the failure of NO_2 to engage in self-reactions and produce nitrite. No nitrite was detected above the amount (10%) generated in the heterolysis step, see Scheme 2.

The transient L^2RhO^{2+} in Scheme 2 is analogous to the cobalt complex, L^2CoO^{2+}, a proposed intermediate in a modified Fenton reaction between L^2CoOOH^{2+} and Fe_{aq}^{2+}. In that work, a large excess of Fe_{aq}^{2+} was required to trap all the L^2CoO^{2+} in competition with an intramolecular process that yielded a ligand-modified product, eqs 9−10, where ($L^{2\,\bullet+}$) represents a coordinated ligand radical cation.[39]

$$L^2CoOOH^{2+} + Fe_{aq}^{2+} \rightarrow L^2CoO^{2+} + Fe_{aq}OH^{2+} \quad (9)$$

$$L^2CoO^{2+} \xrightarrow{Fe_{aq}^{2+},\ H^+} L^2Co^{3+} + H_2O$$

$$L^2CoO^{2+} \rightarrow [(L^{2\,\bullet+})Co^{III}O] \rightarrow \text{Products} \quad (10)$$

The reaction between L^2RhOOH^{2+} and Fe^{2+} in the present work exhibited a clean 2:1 stoichiometry under all the conditions employed, suggesting that the lifetime of L^2RhO^{2+} may be longer than that of L^2CoO^{2+}. We are currently exploring the chemistry of both of these species.

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