

# Final Technical Report

Grant #: DE-FG07-98ER14905

Title: Reactivity of Peroxynitrite: Implications for Hanford Waste Management and Remediation

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Total project period: 09/15/98-09/14/01

## Research summary:

### *1. General objectives*

The purpose of this grant has been to provide basic chemical research in support of a major project undertaken at Brookhaven National Laboratory (BNL) whose purpose was to provide better understanding of the complex chemical processes occurring in nuclear storage tanks on the Hanford reservation. More specifically, the BNL grant (EMSP #73824, Sergei V. Lymar, PI) was directed at evaluating the extent of radiation-induced formation of peroxynitrite anion (ONOO<sup>-</sup>) in the tanks and its possible use in chemical remediation schemes. In its original submission, grant DE-FG07-98ER14905 was incorporated as a subcontract in EMSP 73824, but was later changed to an independent grant to avoid unnecessary duplication of administrative support (i.e., at both WSU and BNL).

Two research projects were carried out under the auspices of this grant comprising:

- (1) mechanistic studies directed at evaluating the extent of generation of radical species ( $\cdot\text{OH}$ ,  $\cdot\text{NO}_2$ ) during peroxynitrite decay
- (2) kinetic analyses of the oxidation of chromic species to chromate ion by peroxo compounds

As discussed in the original EMSP proposal (EMSP #73824, Sergei V. Lymar, PI), the first study is crucial to understanding potential peroxynitrite-initiated reaction sequences in the mixed-waste Hanford tanks and the second is relevant to remediation strategies that would improve long-term storage of the wastes by removing chromic polymers that interfere with vitrification. Results from the supporting studies done in my lab under the auspices of ER14905 are summarized below.

## 2. Secondary radical formation during peroxyxynitrite decay

Protonation or formation of Lewis acid adducts of  $\text{ONOO}^-$  leads to its rapid isomerization to  $\text{NO}_3^-$  or decomposition to  $\text{NO}_2^-$  plus  $\text{O}_2$  in reactions that are highly dependent upon medium conditions. Many of the chemical oxidations carried out by peroxyxynitrite involve secondary species produced during decomposition as the actual oxidants. The identities of these secondary oxidants have been hotly disputed for nearly a decade, but identifying them is clearly essential to understanding the overall chemical reactivity of peroxyxynitrite. In earlier work, we established that  $\text{CO}_2$ -catalyzed peroxyxynitrite decay proceeded via formation of a  $\text{ONOO}^- \text{CO}_2^-$  adduct that underwent homolysis of its peroxy O-O bond to yield  $^{\bullet}\text{NO}_2$  and  $^{\bullet}\text{CO}_3^-$  as secondary oxidants. Although we have also shown that analogous decay of the  $\text{ONOOH}$  via O-O homolysis to yield  $^{\bullet}\text{NO}_2$  and  $^{\bullet}\text{OH}$  is consistent with all of the known chemistry of the relevant species, this view has been strongly opposed by others who, for various reasons, deny that  $\text{ONOOH}$  can be a precursor to  $^{\bullet}\text{OH}$ . To resolve this issue, we have designed and carried out experiments to distinguish between the homolysis mechanism and mechanisms by others that do not involve intermediary formation of radicals. The results, presented in the appended manuscript *i*, are totally consistent with the homolysis mechanism, but exclude the other mechanisms from consideration. This work is important because it places the notion that  $\text{ONOOH}$  spontaneously decomposes to generate these strongly oxidizing radicals on a firm foundation.

## 3. Chromium(III) oxidation by potassium monoperoxide ("oxone")

The purpose of these studies was to provide comparison of the reactivity of  $\text{ONOO}^-$  with that of a second peroxide that, unlike peroxyxynitrite, was relatively stable in the absence of reductants, yet retained its strongly oxidizing properties. The kinetics of oxidation of Cr(III) by oxone ( $2\text{KHSO}_5 \cdot \text{KHSO}_4 \cdot \text{K}_2\text{SO}_4$ ) in strongly alkaline media (mimicking the Hanford tank environment) were carried out by adding acidic  $\text{Cr}(\text{NO}_3)_3$  solutions to an excess of oxone in 2.5 M NaOH. Under these conditions, the oxone has a decay half-time of several hours at room temperature, as determined by iodometric titration. For kinetic runs, however, reagent solutions of oxone were prepared in 0.1 M acetic acid, pH 4.5, a medium in which they were considerably more stable. (For example, an oxone solution with 3 mmol/L oxidizing capacity lost only ~ 10% of its titer after several days storage at 10° C.) Stock Cr(III) solutions were prepared by dissolving  $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$  in 0.1 M  $\text{HNO}_3$ ; these were standardized by digesting the complex ion in strongly alkaline solution containing  $\text{HO}_2^-$ . Under these conditions, Cr(III) species are quantitatively converted to  $\text{CrO}_4^{2-}$ , whose concentration can be determined by spectrophotometric titration. Kinetic runs were made by adding Cr(III) to 2.5 M NaOH, followed immediately by a stoichiometric excess of oxone. Under all conditions investigated, a complex kinetic waveform for  $\text{CrO}_4^{2-}$  formation was observed, comprising at least three temporally resolved steps. Relative amplitudes and apparent reaction half-times for these steps were insensitive to the manner in which Cr(III) was added to the basic solution and to the reagent concentrations over the range,  $[\text{Cr}(\text{III})] = 20\text{-}50 \mu\text{M}$ ,  $[\text{oxone}] = 1\text{-}8 \text{ mM}$ . Approximate half-times for the three steps were:  $t_{1/2}(1) \approx 2 \text{ min}$ ,  $t_{1/2}(2) \approx 16 \text{ min}$ , and  $t_{1/2}(3) \approx \text{very slow (hours)}$ ; for most runs, the intermediate pathway

constituted ~ 50% of the total added Cr(III). The likely explanation for this behavior is that Cr(III) undergoes hydrolytic polymerization to less reactive species in competition with its oxidation to  $\text{CrO}_4^{2-}$ . Nonetheless, the absence of dependence of rates upon [oxone] indicates that reactions of all of the species are complex. These data have not been published, but have been forwarded to Dr. Lymar for inclusion in his grant reports (i.e., EMSP #73824).

#### 4. *Other activities*

Although this grant has expired, Dr. Lymar and I continue to informally collaborate on some of the project goals. Most notably, we are currently jointly writing an invited review of peroxyxynitrite chemistry for publication in *Chemical Reviews*. This manuscript will be completed by early spring.

#### 5. *Publications emanating from this grant:*

- (1) S. V. Lymar, R. F. Khairutdinov, and J. K. Hurst: "Hydroxyl Radical Formation by O-O Bond Homolysis in Peroxynitrous Acid" *Inorg. Chem.* **42**, 5259-5266 (2003). (appendcd)
- (2) S. V. Lymar and J. K. Hurst: "The Chemistry of Peroxynitrite and Peroxynitrous Acid", *Chem. Revs.*, in preparation.

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## **Hydroxyl Radical Formation by O–O Bond Homolysis in Peroxynitrous Acid**

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## Hydroxyl Radical Formation by O–O Bond Homolysis in Peroxynitrous Acid

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Peroxynitrite decay in weakly alkaline media occurs by two concurrent sets of pathways which are distinguished by their reaction products. One set leads to net isomerization to  $\text{NO}_3^-$  and the other set to net decomposition to  $\text{O}_2$  plus  $\text{NO}_2^-$ . At sufficiently high peroxynitrite concentrations, the decay half-time becomes concentration-independent and approaches a limiting value predicted by a mechanism in which reaction is initiated by unimolecular homolysis of the peroxy O–O bond, i.e., the following reaction:  $\text{ONOOH} \rightarrow \cdot\text{OH} + \cdot\text{NO}_2$ . This dynamical behavior excludes alternative postulated mechanisms that ascribe decomposition to bond rearrangement within bimolecular adducts. Nitrate and nitrite product distributions measured at very low peroxynitrite concentrations also correspond to predictions of the homolysis model, contrary to a recent report from another laboratory. Additionally, (1) the rate constant for the reaction  $\text{ONOO}^- \rightarrow \cdot\text{NO} + \cdot\text{O}_2^-$ , which is critical to the kinetic model, has been confirmed, (2) the apparent volume of activation for ONOOH decay ( $\Delta V^\ddagger = 9.7 \pm 1.4 \text{ cm}^3/\text{mol}$ ) has been shown to be independent of the concentration of added nitrite and identical to most other reported values, and (3) complex patterns of inhibition of  $\text{O}_2$  formation by radical scavengers, which are impossible to rationalize by alternative proposed reaction schemes, are shown to be quantitatively in accord with the homolysis model. These observations resolve major disputes over experimental data existing in the literature; despite extensive investigation of these reactions, no verifiable experimental evidence has been advanced that contradicts the homolysis model.

## Introduction

Interest in the chemical reactivity of peroxynitrous acid (ONOOH) has been rekindled by recognition that this powerful oxidant<sup>1</sup> could be formed in respiring tissues by radical coupling of  $\cdot\text{O}_2^-$  and  $\cdot\text{NO}$  and might thereby be a major contributor to diseases associated with oxidative stress and cellular mechanisms of host resistance to pathogenic organisms.<sup>2</sup> The oxidative behavior of ONOOH is highly

unusual in the sense that many of its reactions are first-order in ONOOH and independent of the reductant concentration. Further, stoichiometric oxidation of the other reactant is never achieved in these reactions, with a significant portion of the product mixture being the ONOOH isomerization product, nitric acid. These properties indicate that oxidation requires rate-limiting conversion of ONOOH into secondary species that are the actual oxidants.<sup>3</sup>

The formation of radical intermediates in oxidations of simple aromatic compounds was evident 50 years ago from the appearance of diaryl compounds in the product mixtures,<sup>4</sup> and an early kinetic study established that decomposition to nitric acid was consistent with intermediary formation of  $\cdot\text{OH}$ .<sup>5</sup> Hydroxyl radical was again suggested as the reactive species in a seminal paper that drew attention to the possible

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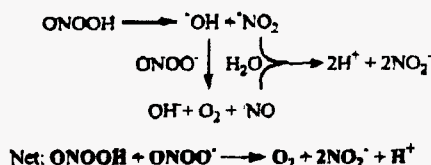
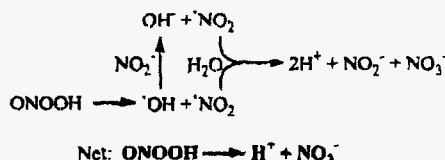
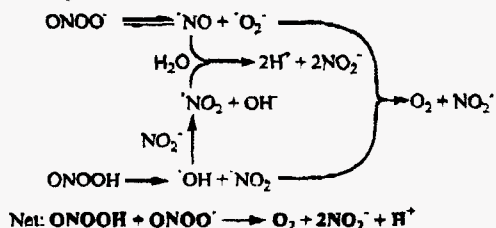
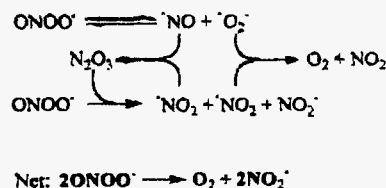
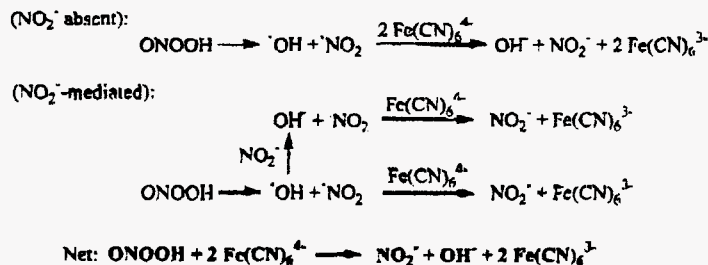
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Scheme 1. Radical Pathways for Peroxynitrite Decay<sup>a</sup>

i) direct decomposition:

ii)  $\text{NO}_2^-$ -mediated isomerization:iii)  $\text{NO}_2^-$ -mediated decomposition:iv)  $\text{N}_2\text{O}_3$ -catalyzed decomposition:v)  $\text{Fe}(\text{CN})_6^{4-}$  inhibition of  $\text{O}_2$  formation:

<sup>a</sup> Homolysis of the O–O bonds in ONOOH leads to initial formation of geminate [ $\cdot\text{NO}_2$ ,  $\cdot\text{OH}$ ] radical pairs, which then either (1) recombine to regenerate ONOOH, (2) isomerize within the solvent cage to give  $\text{HNO}_3$ , or (3) escape the cage as radicals to undergo bimolecular reactions with other solution components. For clarity, only those reactions that follow cage escape are shown, as the caged radicals are too short-lived to engage in reactions other than with themselves. Only ~28% of the total ONOOH undergoing decay leads to formation of these cage-separated radicals.<sup>26,27</sup> Although not shown, isomerization of ONOOH to  $\text{H}^+ + \text{NO}_3^-$  is included in the mechanistic analysis of the data according to the homolysis model (as outlined in the Supporting Information).

biological significance of peroxynitrite.<sup>6</sup> However, this view was quickly abandoned when thermodynamic estimates suggested that the O–O bond in ONOOH was too strong to account for the rate of first-order oxidations by ONOOH; i.e., the measured rate constant for these reductant-independent oxidations was greater than that predicted for O–O bond homolysis.<sup>1</sup> An alternative mechanism was proposed which assigned the activation step to cis–trans isomerization of ONOOH with either the trans conformer or an unstable “transoid” intermediate being the reactive species. This model was widely adopted by the biomedical community at that time.

The thermodynamic analysis which formed the basis for rejection of O–O homolysis as the activation step was questioned by Merényi and Lind, who, by using a different set of premises, found that the calculated homolysis rate constant was consistent with measured values.<sup>7</sup> The ensuing debate over the relative merits of the two calculations<sup>8</sup> led us to seek resolution of the issue by examining whether intermediary formation of  $\cdot\text{OH}$  and  $\cdot\text{NO}_2$  could account quantitatively for product distributions obtained from ONOOH decomposition.<sup>9</sup> In acidic media, ONOOH isomerizes quan-

titatively to  $\text{HNO}_3$ ; in neutral to weakly alkaline media, a competing pathway involving decomposition to  $\text{O}_2 + 2\text{NO}_2^-$  is expressed.<sup>9–12</sup> This behavior can be modeled by invoking a fairly complex set of reactions initiated by O–O homolysis; the important components comprising this set are displayed in Scheme 1 (pathways i–iv). Although these pathways are not entirely independent, each can prevail under certain reaction conditions. Because the rate constants for every elementary reaction in these pathways have been independently measured, it is possible in this case to calculate without

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Merényi, G.; Lind, J.; Goldstein, S.; Czapski, G. *Chem. Res. Toxicol.* 1998, 11, 712–713. Very recently, the latter researchers have shown that calculation of the Gibb's energy of formation of ONOOH based upon the kinetically determined equilibrium constant for the reaction,  $\text{ONOOH} + \text{H}_2\text{O} \rightleftharpoons \text{HNO}_2 + \text{H}_2\text{O}_2$  (Merényi, G.; Lind, J.; Czapski, G.; Goldstein, S. *Inorg. Chem.* 2003, 42, 3796–3800) yields the same value as previously estimated by Merényi and Lind from the kinetically determined  $\text{ONOO} \cdot \rightleftharpoons \cdot\text{O}_2^- + \cdot\text{NO}$  equilibrium constant.<sup>14</sup> Thus, their calculations using thermodynamic cycles based upon two distinct reactions yield a self-consistent data set, thereby providing strong support for the O–O homolysis mechanism.  
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introducing any adjustable parameters the product distributions over a wide range of experimental conditions. We found that the model accurately predicted these distributions for all conditions reported in the literature and also accounted for some otherwise puzzling effects of added radical scavengers. We concluded that O-O homolysis is indeed the activation process in first-order reactions of ONOOH.

This conclusion has recently been challenged by Koppenol and Kissner, who have examined the product distributions over a wider range of experimental conditions.<sup>13</sup> Although their results are generally in agreement with earlier published data,<sup>9,10</sup> they find that, in alkaline solution at very low peroxynitrite concentrations, the measured yield of NO<sub>2</sub><sup>-</sup> is less than predicted by the radical model. Indeed, if these data are correct, the radical model would have to be modified (in a way that is not obvious) or abandoned. Koppenol and co-workers have also challenged the model on a second point, namely, the rate constant used for the reaction step involving dissociation of peroxynitrite anion, i.e., ONOO<sup>-</sup> → \*NO + \*O<sub>2</sub><sup>-</sup>. This rate constant, which is critical to the calculations, was measured indirectly by Merényi and Lind by observing the rate of formation of the nitroform anion, C(NO<sub>2</sub>)<sub>3</sub><sup>-</sup>, in alkaline solutions containing the \*O<sub>2</sub><sup>-</sup> scavenger, tetranitromethane, C(NO<sub>2</sub>)<sub>4</sub>.<sup>14</sup> Koppenol and co-workers reported that C(NO<sub>2</sub>)<sub>3</sub><sup>-</sup> formation was not inhibited when the medium also contained excess \*NO, which is inconsistent with the mechanism proposed by Merényi and Lind.<sup>15</sup> However, in reexamining this experiment, Merényi, Goldstein, and co-workers found that under an atmosphere of \*NO the reaction of ONOO<sup>-</sup> with C(NO<sub>2</sub>)<sub>4</sub> was completely inhibited, as the mechanism predicted.<sup>16</sup>

Since the whole issue of significant radical formation by ONOOH now rests upon resolving experimental discrepancies between laboratories, we have undertaken a careful reexamination of the points in dispute. Our findings, reported herein, remain totally consistent with the radical mechanism. Moreover, we present arguments on the basis of dynamical and stoichiometric evidence that eliminate from consideration an alternative mechanism proposed to account for ONOOH decomposition to O<sub>2</sub> and NO<sub>2</sub><sup>-</sup>.<sup>13</sup>

## Experimental Section

**Materials.** Alkaline peroxynitrite solutions were prepared by three different methods and stored frozen at -80 °C until used. Flow-mixing of NaNO<sub>2</sub> with acidic solutions of H<sub>2</sub>O<sub>2</sub> followed by an alkaline quenching according to the explicit procedures of Saha et al. gave ~100 mM ONOO<sup>-</sup> solutions that also contained ~20 mM NO<sub>2</sub><sup>-</sup>.<sup>17</sup> Ozonolysis of 0.1 M NaN<sub>3</sub> at pH 12 (NaOH) using a home-built ozonizer gave solutions that accumulated maximally 50–60 mM ONOO<sup>-</sup>, which then declined upon further exposure to O<sub>3</sub>, essentially as reported by Uppu et al.<sup>18</sup> To minimize residual

N<sub>3</sub><sup>-</sup> content,<sup>18</sup> ozonolysis was carried out until the ONOO<sup>-</sup> concentration had dropped to 25–30 mM; these solutions also contained ~20 mM NO<sub>2</sub><sup>-</sup>. Frozen solutions of tetramethylammonium peroxynitrite, synthesized according to Bohle et al.,<sup>19</sup> were kindly provided by Prof. Koppenol, ETH, Zurich. Decomposition of these peroxynitrite solutions during melting was minimized by addition of a small amount of NaOH to the frozen pellet before warming the sample. The solutions so obtained were ~20 mM in ONOO<sup>-</sup> and contained ~4 mM NO<sub>2</sub><sup>-</sup>. To assess the short-term stabilities of the various preparations, decomposition rates of dilute ONOO<sup>-</sup> were briefly investigated at pH 12 and 23 °C. For all the preparations, decomposition was approximately linear in time over the span of 1–2 h: the rates of ONOO<sup>-</sup> loss in 5 mM samples prepared from H<sub>2</sub>O<sub>2</sub>/NaNO<sub>2</sub> and O<sub>3</sub>/NaN<sub>3</sub> were ~10%/h, and that in 0.4–0.8 mM (CH<sub>3</sub>)<sub>4</sub>N(ONOO) was ~20%/h. Addition of 0.67 mM diethylenetriaminepentaacetic acid did not affect the (CH<sub>3</sub>)<sub>4</sub>N(ONOO) decomposition rate. As indicated in subsequent sections, all preparations gave equivalent results.

**Methods.** The kinetics of C(NO<sub>2</sub>)<sub>3</sub><sup>-</sup> formation from C(NO<sub>2</sub>)<sub>4</sub> were measured in an all-glass apparatus designed specifically to rigorously exclude O<sub>2</sub>. This apparatus consisted of a round 50 mL mixing chamber connected via a 4-way V-bore stopcock to an optical cuvette.<sup>20</sup> The system was purged by percolating gases through the reaction medium: by rotating the stopcock 90°, the purging gas pressure could be used to drive the solution from the mixing chamber into the cuvette. The apparatus was also fitted with a double-septum antechamber through which anaerobic reactant solutions could be introduced using standard syringe-transfer techniques; the double-septum arrangement prevented adventitious O<sub>2</sub> from being carried into the reaction compartment during piercing of the septa. In a typical experiment, the reaction medium was first purged to obtain an atmosphere of N<sub>2</sub> or \*NO, and then ONOO<sup>-</sup> and C(NO<sub>2</sub>)<sub>4</sub> were added in that order. Immediately following the last addition, the optical cuvette was loaded and C(NO<sub>2</sub>)<sub>3</sub><sup>-</sup> formation was monitored at 350 nm using a conventional diode array spectrophotometer (Hewlett-Packard 8452A). The time required to mount the cuvette was ~30 s, so for the fastest reactions studied the first ~30% of the reaction was not recorded. Dinitrogen trioxide was removed from commercial \*NO (AGA Specialty Gas, Cleveland, OH) by slowly passing the gas through two 0.5 M NaOH scrubbing towers, followed by a tower containing H<sub>2</sub>O. The efficiencies of the scrubbers were checked by cycling N<sub>2</sub>/\*NO/N<sub>2</sub> through the reaction medium in the apparatus under conditions that duplicated those used for the kinetics experiments and then measuring the amount of accumulated NO<sub>2</sub><sup>-</sup> using the standard Griess colorimetric assay.<sup>21</sup> Whereas accumulation of NO<sub>2</sub><sup>-</sup> in the first NaOH scrubber was extensive (~1 mM), the amounts in the second and third scrubbers were negligible and the amount in the reaction medium was below detectable limits (≤5 μM).

The yield of NO<sub>2</sub><sup>-</sup> formed during alkaline decomposition of ONOOH was determined by measuring accumulated NO<sub>2</sub><sup>-</sup> when known amounts of the peroxide were decomposed in anaerobic buffers prepared from NaH<sub>2</sub>PO<sub>4</sub> (pH 4.5) or Na<sub>2</sub>HPO<sub>4</sub> (pH 9.3). For each such analysis, serial additions of NO<sub>2</sub><sup>-</sup> that had been standardized spectrophotometrically (ε<sub>352</sub> = 24.7 M<sup>-1</sup> cm<sup>-1</sup>) were

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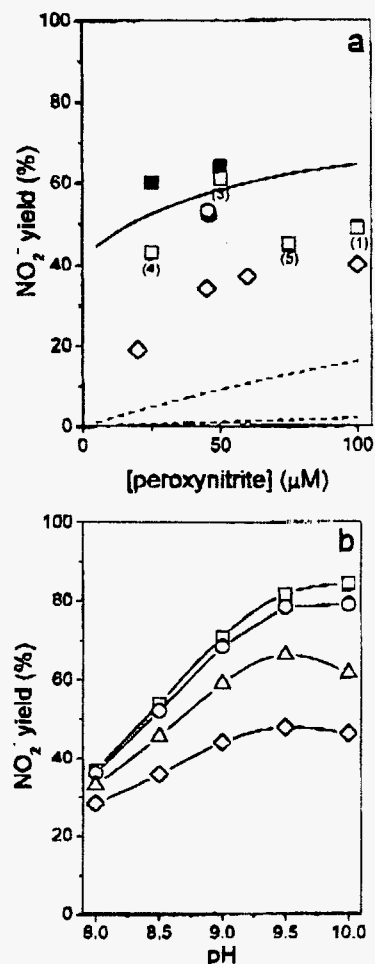
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and  $\text{NO}_2^-$  obtained from decomposition of peroxynitrite at low concentrations in alkaline solutions are significantly less than predicted by the radical model.<sup>13</sup> Upon reinvestigating this point using peroxynitrite solutions prepared by reaction between  $\text{H}_2\text{O}_2$  and  $\text{NaNO}_2$ , by  $\text{N}_3^-$  ozonolysis, and in the Koppenol laboratory from solid tetramethylammonium peroxynitrite, we find no discrepancy between experimentally determined and predicted yields. In these studies, several different  $\text{H}_2\text{O}_2$ - $\text{NaNO}_2$  preparations were used, all of which gave equivalent results;  $\text{NO}_2^-$  yields measured in samples that had been stored at  $-80^\circ\text{C}$  for up to 1 year were unchanged from yields obtained on the same samples shortly after preparation, indicating that long-term storage had no effect upon the reaction stoichiometry, as should be expected. The total amounts of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  present in product solutions that had decayed at pH 4.5 and 9.3 were identical, indicating that no other nitrogen-containing species were formed. Relevant data from both laboratories are displayed in Figure 2a; also shown (Figure 2b) are results of calculations based upon the radical mechanism (Scheme 1) that predict that the fractional yields will be insensitive to pH in the measured region but increase slowly with increasing peroxynitrite concentrations toward an asymptotic limit of  $\sim 85\%$ . Also, at 1.2 mM total peroxynitrite, the measured nitrite yield was 69%, in accord with predictions based upon Scheme 1 (Figure 2b) and with our earlier results based upon  $\text{O}_2$  measurements.<sup>9</sup> The  $\text{NO}_2^-$  yields were independent of ionic strength over the measured phosphate concentration range of 50–500 mM; very similar yields of 42% at 25  $\mu\text{M}$  peroxynitrite and 40% at 50  $\mu\text{M}$  peroxynitrite were obtained in 50 mM  $\text{NH}_3/\text{NH}_4\text{NO}_3$  buffer, pH 9.3. Our data are therefore quantitatively consistent with predictions based upon the radical mechanism over the entire experimental range that has been reported. They are also consistent with recent experimentally determined yields of secondary oxidants generated by  $\text{ONOOH}$  decomposition,<sup>26–28</sup>

**Pressure Dependence of Peroxynitrite Reactions.** Homolysis of  $\text{ONOOH}$  to two neutral fragments is expected to give rise to a significant increase in the molar volume of the transition state ( $\Delta V^\ddagger$ ), so that reactions initiated by O-O homolysis will be inhibited at high pressures. The Koppenol laboratory originally reported  $\Delta V^\ddagger = 1.7 \text{ cm}^3/\text{mol}$  for  $\text{ONOOH}$  decomposition, from which they argued that the reaction did not occur by homolysis;<sup>29</sup> they have subsequently revised this number upward.<sup>30</sup> Reinvestigations by other groups<sup>22,31</sup> have placed  $\Delta V^\ddagger$  at  $\approx 10 \text{ cm}^3/\text{mol}$  for this reaction and for indirect oxidations (e.g., of  $\text{Fe}(\text{CN})_6^{4-}$ ) by  $\text{ONOOH}$ . In contrast, direct bimolecular reactions involving  $\text{ONOOH}$  exhibit negative values for  $\Delta V^\ddagger$  which are dependent upon the reductant identities.<sup>22</sup> Relevant data are displayed in



**Figure 2.** Nitrite yields from peroxynitrite decay in alkaline solutions. Panel a: measured yields at  $22^\circ\text{C}$  in 0.1 M disodium hydrogen phosphate, pH 9.3. Solid squares (automated) and open squares (manual) indicate Griess analyses of reagent prepared by reaction between  $\text{H}_2\text{O}_2$  and  $\text{NaNO}_2$  (given in parentheses are the numbers of individual determinations made using separate preparations, with an estimated error of  $\pm 10\%$ ). The solid circle indicates manual Griess analysis of reagent prepared by azide ozonolysis,<sup>18</sup> and the open circle indicates manual Griess analysis of a solution of tetramethylammonium peroxynitrite. The open diamonds represent data reported by Kissner and Koppenol for pH 9.0.<sup>13</sup> The solid line presents the calculated yield at pH 9.3 for these conditions on the basis of the radical mechanism in Scheme 1. Dashed lines give calculated yields for the bimolecular pathway at pH 9.0 according to Scheme 3 with  $K_1 = 1.6 \times 10^{-7} \text{ M}$ ,  $k_1 = 1.2 \text{ s}^{-1}$ , and  $K_A k_4 = 2.5 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$  (upper line) or  $K_A k_4 = 2.5 \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$  (lower line). Panel b: pH and concentration dependence for nitrite yields in this region, as predicted by the radical mechanism according to Scheme 1. Peroxynitrite concentrations are the following: 10  $\mu\text{M}$  (diamonds); 100  $\mu\text{M}$  (triangles); 500  $\mu\text{M}$  (circles); 1000  $\mu\text{M}$  (squares).

Figure 3; as discussed in the original papers,<sup>22,31</sup> the larger  $\Delta V^\ddagger$  value is fully consistent with O-O bond homolysis being the  $\text{ONOOH}$  activation step.

In our earlier investigation of the pressure dependence of  $\text{ONOOH}$  decomposition we reported an apparent dependence of  $\Delta V^\ddagger$  upon the  $\text{NO}_2^-$  concentration.<sup>22</sup> This effect cannot be accommodated by the simple radical mechanism given in Scheme 1. Reinvestigation of this reaction over a wider range of conditions has shown that the effect was artifactual and that  $\Delta V^\ddagger$  is independent of  $[\text{NO}_2^-]$  in accord with the model (Figure 3 inset).

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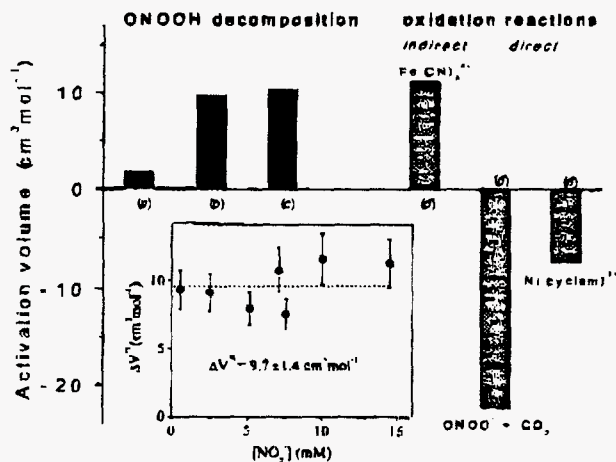
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(30)  $\Delta V^\ddagger = 6-7 \text{ cm}^3/\text{mol}$  (W. H. Koppenol, personal communication).

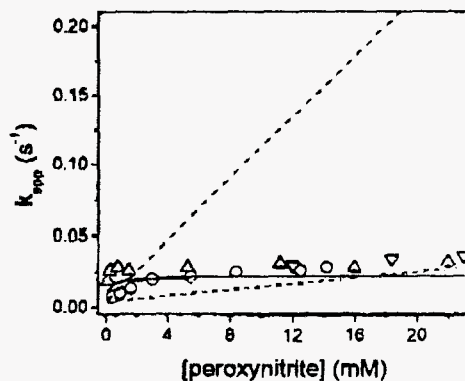
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**Figure 3.** Pressure dependence of peroxyxynitrite reactions. Data are from the following: (a) ref 29; (b) this work; (c) ref 31; (d) ref 22. The inset gives the dependence of  $\Delta V^\ddagger$  upon  $[\text{NO}_2^-]$  measured in this work for decomposition of 0.35 mM ONOOH in 0.15 M sodium phosphate, pH 4.5, and 20 °C.

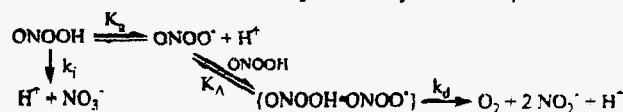
### Inadequacies of Other Proposed Reaction Mechanisms.

At pH 8–11, where decomposition into  $\text{NO}_2^-$  and  $\text{O}_2$  is the dominant pathway, the kinetics of peroxyxynitrite decay exhibit nonexponential behavior and pronounced medium effects<sup>29</sup> whose origins have not been conclusively identified.<sup>32</sup> Consequently, full mechanistic analysis of the decay kinetic profiles is rendered equivocal. Although significant, the deviations from exponentiality are not dramatic and the experimentally determined peroxyxynitrite half-life,  $t_{1/2}$ , gives a useful quantitative parameter, which can be more conventionally expressed as the apparent rate constant,  $k_{\text{app}} = \ln 2/t_{1/2}$ , and used for comparing predictions of different models with the kinetic data. The radical mechanism clearly predicts that at high concentrations the apparent rate constant for decay will become independent of the initial peroxyxynitrite concentration ( $C_0$ ). As required by this model,  $k_{\text{app}}$  became independent of  $C_0$  at sufficiently high peroxyxynitrite concentrations (Figure 4). Furthermore, the model fairly accurately gives the limiting value for  $k_{\text{app}}$ . Under the chosen experimental conditions, medium effects were negligible.<sup>32</sup> Specifically,  $k_{\text{app}}$  was independent of the buffer identity (phosphate vs ammonium) and concentration over a severalfold range (Figure 4). No evidence of metal ion



**Figure 4.** Apparent rate constants (as defined in the text) for peroxyxynitrite decay in various media. Conditions: 0.06 M disodium hydrogen phosphate, pH 9.3 (circles); 0.012 M disodium hydrogen phosphate, pH 9.3 (inverted triangles); 0.05 M ammonia/ammonium nitrate, pH 9.4 (upright triangles); all reactions at 22 °C. The solid line shows the dependence predicted by the radical mechanism according to Scheme 1; dashed lines show the dependence predicted for reaction according to Scheme 3 using  $K_A$  and  $k_d$  given in Figure 2a and  $K_A k_d = 2.5 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$  (upper line) or  $K_A k_d = 2.5 \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$  (lower line).

### Scheme 3. Bimolecular Pathway for Peroxyxynitrite Decay<sup>13</sup>



catalysis of  $\text{ONOO}^-$  decay was found; addition of 1 mM of the chelators diethylenetriaminepentaacetic acid or bathocuproin disulfonate to the phosphate buffers, passage of buffer solutions down Chelex-100 cation exchange columns, and use of purified water and buffer salts from various sources had no effect on the decay profiles.

Koppenol and co-workers have proposed that decomposition of peroxyxynitrite to  $\text{O}_2$  and  $\text{NO}_2^-$  proceeds through a second-order reaction involving association of ONOOH and  $\text{ONOO}^-$  to form a cyclic dimer that subsequently undergoes concerted bond rearrangement (Scheme 3);<sup>13</sup> this mechanism is generally accepted as one of the bimolecular pathways for decomposition of organic and inorganic peroxides.<sup>33</sup> However, unlike these peroxides, ONOOH also decays by concurrent isomerization to nitric acid. Consequently, the rate law predicted by Scheme 3 for peroxyxynitrite decay is more complex than the simple second-order rate laws that describe decomposition of other peroxides. The analytical solution to the rate law for the decay reaction given in Scheme 3 is

$$C_t = C_0 \frac{\exp(-k_1 t)}{1 + P\{1 - \exp(-k_1 t)\}}$$

where  $C_0 = [\text{ONOO}^-]_0 + [\text{ONOOH}]_0$  is the initial concentration of peroxyxynitrite not bound in the dimer,  $C_t$  is the

(32) The radical model predicts that high levels of phosphate ions can be rate-retarding under the experimental conditions. This follows because  $\text{N}_2\text{O}_3$ , formed during peroxyxynitrite decomposition, reacts rapidly with  $\text{ONOO}^-$ , setting up a catalytic cycle for further decomposition (Scheme 1, pathway iv). The magnitude of the contribution of pathway iv to peroxyxynitrite decay is determined by the relative rates of reaction of  $\text{N}_2\text{O}_3$  with peroxyxynitrite anion and solvent, i.e., the following reaction:  $\text{N}_2\text{O}_3 + \text{H}_2\text{O} \rightarrow 2\text{H}^+ + 2\text{NO}_2^-$ . Under highly alkaline conditions, hydrolysis of  $\text{N}_2\text{O}_3$  by  $\text{OH}^-$  will be the dominant reaction.<sup>34</sup> Thus, pathway iv will contribute to peroxyxynitrite decay only in weakly alkaline solutions (pH = 7–12). Earlier work on  $\text{N}_2\text{O}_3$  nitrosation reactions<sup>35,36</sup> has indicated that  $\text{N}_2\text{O}_3$  hydrolysis could be catalyzed by phosphate. At sufficiently high concentrations, phosphate will therefore effectively remove  $\text{N}_2\text{O}_3$  via hydrolysis, decreasing  $\text{N}_2\text{O}_3$ -catalyzed peroxyxynitrite decay via pathway iv and thereby decreasing the overall decay rate in weakly alkaline media. Because the rate constant for reaction between phosphate and  $\text{N}_2\text{O}_3$  is not known at pH 9, this reaction was not included in the lifetime calculations. This omission appears justified on the basis that the experimentally determined values were insensitive to both phosphate buffer concentration at low ionic strengths and the identity of the buffer (Figure 4).

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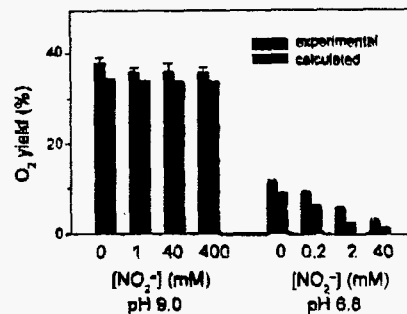
corresponding current concentration, and  $P = 2k_2C_0/k_1$  is a dimensionless parameter that describes the partitioning between the bimolecular decomposition and unimolecular decay pathways. In this equation,  $k_1$  is the rate constant for ONOOH isomerization under the prevailing medium conditions, given by  $k_1 = k_i/(1 + K_a/[H^+])$ , where  $k_i$  is the pH-independent isomerization rate constant and  $K_a$  is the ONOOH acid dissociation constant; similarly,  $k_2 = K_A k_d / \{(1 + K_a/[H^+])(1 + [H^+]/K_a)\}$ , where  $k_d$  is the rate constant for decomposition of the dimer formed between ONOOH and ONOO<sup>-</sup> and  $K_A$  is the dimer formation constant. From the integrated form of the rate law, one obtains

$$k_{app} = \frac{\ln 2}{t_{1/2}} = k_1 \frac{\ln 2}{\ln(2 + P) - \ln(1 + P)}$$

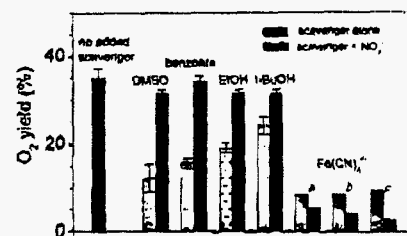
for the apparent rate constant of peroxynitrite decay and  $Y_{nitrite} = 1 - \ln(1 + P)/P$ , for the nitrite yield, defined as  $Y_{nitrite} = [NO_2^-]_{\infty}/C_0$ .

Although there are four fundamental constants in this model, two of them ( $k_i$  and  $K_a$ ) are well established from independent studies; furthermore, the other two ( $k_d$  and  $K_A$ ) are mutually dependent in the sense that only their product ( $K_A k_d$ ) determines  $k_2$  and, therefore,  $P$ , which in turn determines the magnitude of  $NO_2^-$  yields and the concentration dependence of the peroxynitrite decay half-lives. As the following considerations will show, there exists no value for  $K_A k_d$  that can simultaneously account (even approximately) for  $NO_2^-$  yields at low peroxynitrite concentrations and decay rates at high peroxynitrite concentrations. Consequently, one must conclude that the decay mechanism presented in Scheme 3 is in irreconcilable contradiction with the data and, thus, is incorrect. The apparent rate constant for peroxynitrite decay at high reactant concentrations can be approximated (although rather poorly) by the Scheme 3 reaction model when  $K_A k_d = 2.5 \times 10^2 \text{ M}^{-1} \text{ s}^{-1}$  (Figure 4, lower dashed line). However, using these constants, the  $NO_2^-$  yields at low reactant concentrations are calculated to be negligible, in marked contrast to measured yields (Figure 2a, lower dashed line). Even modestly increasing  $K_A k_d$  to  $2.5 \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$  in an attempt to improve the "fit" to the yield data (Figure 2a, upper dashed line) generates a very strong concentration dependence for the decay rate at high peroxynitrite concentrations (Figure 4, upper dashed trace) that is at odds with the rate data. When proposing parameters for Scheme 3, Koppenol and co-workers indicated that these parameters "may be refined".<sup>13</sup> However, it is evident that no refinement is possible, as no single combination of  $K_A$  and  $k_d$  will be found that can satisfy both sets of data for the yields and the rates in Figures 2 and 4. In contrast, the radical model presented in Scheme 1 (for which there are no adjustable parameters) adequately accounts for both rate and yield data over the entire experimentally accessible range (Figures 2a and 4, solid lines).

Finally, we note that bimolecular decomposition mechanisms such as that shown in Scheme 3 also cannot explain the complex effects of added redox reagents upon the product distributions, although these effects are quantitatively pre-



**Figure 5.** Nitrite dependencies of  $O_2$  yields.<sup>9</sup> Conditions: 0.55 mM peroxynitrite decomposed in 0.3 M sodium phosphate at 21 °C. Calculated yields are based upon the radical mechanism presented in Scheme 1. Predicted  $NO_2^-$  yields are twice the measured  $O_2$  yields. The bimolecular mechanism of Scheme 3 predicts nitrite-independent  $O_2$  yields of 24% and 17% for pH 9.0 and 6.8, respectively, under these conditions.



**Figure 6.** Influence of radical scavengers upon  $O_2$  yields.<sup>9</sup> Reaction conditions: all reactions at pH 9.0; other conditions as in Figure 5. When present, organic scavenger and  $NO_2^-$  concentrations were 1.0 mM, except that, with *t*-BuOH,  $[NO_2^-] = 0.5 \text{ mM}$  when added. For organic scavengers, both black and shaded bars are experimental. The data for  $Fe(CN)_6^{4-}$  quenching provide comparison between experimental (shaded bar) and calculated (black bar) values on the basis of Scheme 1. For these reactions  $[Fe(CN)_6^{4-}] = 0.5 \text{ mM}$ , with  $[NO_2^-] = 0$  (set a), 0.5 mM (set b), or 5.0 mM (set c).

dicted by the radical mechanism from known, i.e., independently measured, rate constants.<sup>9</sup> Specifically, added  $NO_2^-$  selectively inhibits  $O_2$  production from peroxynitrite decomposition in neutral but not in alkaline solutions (Figure 5). Organic radical scavengers also inhibit peroxynitrite decomposition; this inhibition is reversed in alkaline solutions by addition of  $NO_2^-$  (Figure 6). However, inhibition of decomposition by  $Fe(CN)_6^{4-}$  is not reversed by  $NO_2^-$  (Figure 6). These effects can be explained by the radical mechanism (Scheme 1) as follows: in neutral media,  $NO_2^-$  competes with ONOO<sup>-</sup> for \*OH (pathways i and ii), generating  $NO_3^-$  instead of  $O_2 + NO_2^-$ ; under more alkaline conditions (pathway iii), the \*NO<sub>2</sub> formed from reaction between  $NO_2^-$  and \*OH reacts with \*NO or \*O<sub>2</sub> generated by ONOO<sup>-</sup> dissociation rather than another \*NO<sub>2</sub>. The immediate product of the \*NO + \*NO<sub>2</sub> reaction is  $N_2O_3$ , a catalyst for the  $O_2$ -forming pathway iv. Added organic radical scavengers react with \*OH formed by ONOOH peroxy bond homolysis, reducing  $O_2$  yields;  $NO_2^-$  effectively competes with these scavengers for \*OH, restoring the yields (pathway iii). Unlike the organic reductants,  $Fe(CN)_6^{4-}$  reacts rapidly with \*NO<sub>2</sub> as well as \*OH (pathway v), so that its inhibition of  $O_2$  formation cannot be reversed by addition of  $NO_2^-$ . At the concentration levels used,  $Fe(CN)_6^{4-}$  does not react directly with peroxynitrite.<sup>26</sup>

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**Supporting Information Available:** Derivation of the rate law for Scheme 3 and a table of rate constants for elementary steps used in numerical simulations based upon Scheme 1. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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