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Oxidation of Peroxynitrite by Inorganic Radicals: A Pulse Radiolysis Study

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Abstract: Reactivity of the peroxynitrite ion toward a number of inorganic radicals was determined by using the pulse radiolysis technique. The rate constants for the oxidation of the $ONOO^-$ ion by $CO_3^{\bullet -}$, $^{\bullet}N_3$, and $ClO_2^{\bullet}$ radicals were determined from their decay kinetics to be $(7.7 \pm 1.2) \times 10^6$ ($I = 0.6$ M), $(7.2 \pm 0.9) \times 10^8$, and $(3.2 \pm 0.3) \times 10^4$ $M^{-1}\ s^{-1}$, respectively. For the $^{\bullet}OH$ radical, the rate constant of $(4.8 \pm 0.8) \times 10^9$ $M^{-1}\ s^{-1}$ was obtained by using competition kinetic analysis. The oxidation potential of the $ONOO^-$ ion was estimated as 0.8 V from the kinetic data. Although thermodynamically favorable, oxidation of $ONOO^-$ by the $^{\bullet}NO_2$ radical was not observed; an upper limit of 2.5×10^4 $M^{-1}\ s^{-1}$ could be set for this reaction. Contribution from some of these reactions to the decomposition of peroxynitrite in the presence and absence of CO_2 is discussed.

Introduction

Peroxynitrite ($ONOOH/ONOO^-$) is a powerful oxidant that can be produced in biological systems from superoxide and nitric oxide. This reaction is fast ($k = (4.3-6.7) \times 10^9\ M^{-1}\ s^{-1}$)[1,2] and is expected to be efficient at physiological levels of $O_2^{\bullet -}$ and $^{\bullet}NO$. The reactivity of peroxynitrite toward biological molecules[3] and its very high toxicity toward cells[4] are presently under intense investigation as potential causes of a number of debilitating diseases.

Peroxynitrite ion is fairly stable, but its conjugate peroxynitrous acid (ONOOH, $pK_a = 6.8$)[3] decomposes rapidly ($\tau_{1/2} = 0.53$ s at 25 °C); isomerization to nitrate is the major decay route in acidic media. On its way to NO_3^-, a significant portion (∼40%) of ONOOH produces a highly oxidizing intermediate with the reactivity similar to that of the hydroxyl radical.[3,5] It has been suggested that this intermediate can oxidize the $ONOO^-$ ion and that this reaction may be responsible for generation of nitrite and oxygen during peroxynitrite decomposition above pH 5.[6] Lymar and Hurst[7] have shown that the peroxynitrite ion reacts very fast with carbon dioxide, apparently forming the $ONOOCO_2^-$ adduct. We have recently reported that decomposition of this adduct generates reactive intermediates capable of oxidizing organic and inorganic compounds.[8−10] These intermediates have been suggested to be $^{\bullet}NO_2$ and $CO_3^{\bullet -}$ radicals, which are strong oxidants formed by the homolytic cleavage of the peroxo O−O bond of the $ONOOCO_2^-$ adduct.[8−10] At low peroxynitrite concentration and in the absence of oxidizable compounds, the major pathway for the decay of these radicals is their recombination via O^- transfer producing NO_3^- and CO_2.[10,11] However, thermodynamic estimate[12] suggests that the oxidation potential of the $ONOO^-$ ion lies below 1 V, NHE. It is therefore possible that both $^{\bullet}NO_2$ and $CO_3^{\bullet -}$ can also oxidize $ONOO^-$; when the latter is present at high concentration these reactions may effectively compete with radical recombinations.[13]

In summary, substantial evidence has been accumulated that oxidation by both ONOOH and $ONOOCO_2^-$, as well as their decompositions, proceed through formation of strongly oxidizing intermediates. Whether or not these intermediates can, in turn, oxidize their precursor, the $ONOO^-$ ion, thus complicating overall kinetics and mechanisms, remains unexplored. In this study we determine for the first time the rate constants for the reactions of various inorganic radicals, including $CO_3^{\bullet -}$, $^{\bullet}NO_2$, and $^{\bullet}OH$, with $ONOO^-$ and discuss the possible role of these reactions in peroxynitrite decomposition.

Experimental Section

Chemicals. All chemicals were of analytical grade and were used as received. Sodium chlorite (Fluka) contained about 80% $NaClO_2$,

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[12] The gas phase heat of formation of the $ONOO^{\bullet}$ radical was recently estimated by McKee[12a] as $\Delta_f H° = 33$ kcal/mol. From this value and an absolute gas-phase entropy of 68 cal/(mol K)[12b] for this radical we calculate $\Delta_f G°(ONOO_g^{\bullet}) = 42$ kcal/mol. Assuming that terminal oxygen atoms of the $ONOO^{\bullet}$ radical can form 4 hydrogen bonds we estimate its solution free energy as −4 kcal/mol,[12c] which results in $\Delta_f G°(ONOO_{aq}^{\bullet}) = 38$ kcal/mol. From this value and the recent estimate by Merenyi and Lind[12d] for $\Delta_f G°(ONOO_{aq}^-) = 17$ kcal/mol, we obtained $E°(ONOO^{\bullet}/ONOO^-) = 0.9$ V. (a) McKee, M. L. *J. Am. Chem. Soc.* **1995**, *117*, 1629. (b) Guillory, W. A.; Johnston, H. S. *J. Chem. Phys.* **1965**, *42*, 2457. (c) Schwarz, H. A.; Dodson, R. W. *J. Phys. Chem.* **1984**, *88*, 3643. (d) Merenyi, G.; Lind, J. *Chem. Res. Toxicol.* **1997**, *10*, 1246.

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the rest being NaCl and $NaClO_3$. Water for solution preparations was distilled and purified with a Milli-Q purification system.

Fresh solutions of peroxynitrite were prepared daily by reacting nitrite with acidified hydrogen peroxide at room temperature in a quenched-flow system. This system was optimized, as we recently described,[14] to produce the high yield of $ONOO^-$ with only a minor known contamination by residual NO_2^- and H_2O_2. Minimization of these contaminants is crucial for studying oxidation of $ONOO^-$ by free radicals because both NO_2^- and H_2O_2 can compete for these radicals.[13] A Syringe pump (WPI, model SP 230IW) was used to inject either (preparation *A*) 0.63 M $NaNO_2$ and 0.60 M H_2O_2 in 0.7 M $HClO_4$ or (preparation *B*) 0.60 M $NaNO_2$ and 0.60 M H_2O_2 in 0.7 M $HClO_4$ into the first mixing chamber through tangential inlets. The combined solutions were allowed to react in a delay line connected to a second mixing chamber where 3.6 M NaOH was injected with the same flow rate (45 mL/min) to quench the reaction.[14] The yield of $ONOO^-$ depends on the time of quenching[14] and was determined from its absorption at 302 nm with $\epsilon = 1670\ M^{-1}\ cm^{-1}$.[15] The residual H_2O_2 in the stock peroxynitrite solutions was determined iodometrically[16] after diluting these solutions in 0.2 M acetate buffer at pH 4.6 and, in parallel, at pH 2. Because residual NO_2^- destroys H_2O_2 at acidic pH, the yield of I_3^- at pH 2 was used as a blank, which was subtracted from the yield of I_3^- obtained at pH 4.6. It was determined that preparation *A* contained 0.18 M $ONOO^-$ and 0.32−0.49 mM H_2O_2. Preparation *B* contained 0.12 M $ONOO^-$ and 0.94−1.1 mM H_2O_2. These yields of peroxynitrite and residual H_2O_2 are in good agreement with those calculated from kinetic simulation.[14] Specifically, simulation for preparation *A* gave 0.188 M $ONOO^-$, 52 mM NO_3^-, 12.3 mM NO_2^-, and 0.29 mM H_2O_2, while preparation *B* gave 0.122 M $ONOO^-$, 0.117 M NO_3^-, 1.19 mM NO_2^-, and 1.19 mM H_2O_2.

Methods. Pulse radiolysis experiments were carried out mainly in Jerusalem with a 5 MeV Varian 7715 linear accelerator (0.2−1.5 μs electron pulses, 200 mA current). Some experiments were done at Brookhaven with a 2 MeV Van de Graaff accelerator (0.05−0.2 μs pulses). All measurements were made at room temperature in a 4-cm spectrosil cell using three light passes (optical path length 12.1 cm). The yields and decay kinetics of $CO_3^{\bullet-}$, ${}^\bullet NO_2$, ${}^\bullet N_3$, and $ClO_2^\bullet$ radicals were measured from their absorption with $\epsilon_{600} = 1860\ M^{-1}\ cm^{-1}$,[13] $\epsilon_{400} = 200\ M^{-1}\ cm^{-1}$,[13] $\epsilon_{275} = 1690\ M^{-1}\ cm^{-1}$,[17] and $\epsilon_{358} = 1250\ M^{-1}\ cm^{-1}$,[13] respectively.

Results and Discussion

All reactions between $ONOO^-$ and the oxidizing radicals were studied at pH ≥12, where the $ONOO^-$ ion is relatively stable.[3,4] Less than 5% decomposition of peroxynitrite occurred within 15 min after solution preparation, which was the average duration of each experiment.

Carbonate Radical. The rate constants for the reactions of $CO_3^{\bullet-}$ with NO_2^-, H_2O_2, and HO_2^- are 6.6×10^5, 8×10^5, and $(1-5.6) \times 10^7\ M^{-1}\ s^{-1}$, respectively.[13] Concentration of residual H_2O_2 ($pK_a = 11.7$)[18] in the peroxynitrite preparation must therefore be kept as low as possible to minimize interference from HO_2^-. For this reason we used preparation *A* for these experiments as in this preparation contamination by H_2O_2 is less than 0.3% of $ONOO^-$ (see Experimental Section).

Carbonate radical was generated by irradiating N_2O-saturated (~25 mM) aqueous solutions containing 0.2 M sodium carbonate via the following reactions (given in parentheses are the species radiation yields):

$$H_2O \rightarrow e^-_{aq}(2.6),\ {}^\bullet OH\ (2.7),\ H^\bullet\ (0.6),\ H_3O^+\ (2.6) \qquad (1)$$

$$H^\bullet + OH^- \rightarrow e_{aq}^- \qquad k_2 = 2.2 \times 10^7\ M^{-1}\ s^{-1}\ (\text{ref } 19) \qquad (2)$$

$$e^-_{aq} + N_2O \rightarrow N_2 + OH^- + OH^\bullet \qquad k_3 = 9.1 \times 10^9\ M^{-1}\ s^{-1}\ (\text{ref } 19) \qquad (3)$$

$${}^\bullet OH + CO_3^{2-} \rightarrow CO_3^{\bullet-} + OH^- \qquad k_4 = 3.9 \times 10^9\ M^{-1}\ s^{-1}\ (\text{ref } 19) \qquad (4)$$

$$O^{\bullet-} + CO_3^{2-} \rightarrow CO_3^{\bullet-} + OH^- \qquad (5)$$

Although the rate constant for reaction 5 has not been determined,[19] it should be smaller than that for reaction 4 due to electrostatic repulsion. The effective rate constant of the reaction of hydroxyl radicals with CO_3^{2-} is pH dependent and given by

$$k_6 = \frac{k_4[H^+]}{K_{OH} + [H^+]} + \frac{k_5 K_{OH}}{K_{OH} + [H^+]} \qquad (6)$$

where K_{OH} is the acid dissociation constant of the ${}^\bullet OH$ radical ($pK_a = 11.9$).[19]

In the absence of peroxynitrite, the decay of $CO_3^{\bullet-}$ monitored at 600 nm was second order:

$$CO_3^{\bullet-} + CO_3^{\bullet-} \rightarrow \text{products} \qquad (7)$$

with $2k_7 = (3.2 \pm 0.4) \times 10^7\ M^{-1}\ s^{-1}$ at $I = 0.6$ M and $(1.8 \pm 0.2) \times 10^7\ M^{-1}\ s^{-1}$ at $I = 0.04$ M, in agreement with the literature values.[13] We found that addition of up to 0.2 mM NO_3^-, which is the product of the ONOOH isomerization,[3,4] had no effect on the yield and decay rates of $CO_3^{\bullet-}$ at pH 12 (data not shown). The yield of $CO_3^{\bullet-}$ was also unaffected by the addition of up to 1.3 mM of $ONOO^-$, indicating that, under these conditions, carbonate ions scavenged all hydroxyl radicals produced. However, the decay rate of $CO_3^{\bullet-}$ was enhanced in the presence of $ONOO^-$, and changed from second-order to first-order kinetics (Figure 1). When $[ONOO^-]_o \gg [CO_3^{\bullet-}]_o$, the observed first-order rate constant of $CO_3^{\bullet-}$ decay was independent of $[CO_3^{\bullet-}]_o$ and depended linearly on the concentration of added $ONOO^-$ (Figure 2, upper line). Assuming that the observed effect is due to oxidation of $ONOO^-$ by $CO_3^{\bullet-}$,

$$CO_3^{\bullet-} + ONOO^- \rightarrow ONOO^\bullet + CO_3^{2-} \qquad (8)$$

we determined $k_8 = (9.6 \pm 1.4) \times 10^6\ M^{-1}\ s^{-1}$ from the slope of the upper line in Figure 2.

Since the value of k_8 is small, we wanted to verify that the observed acceleration of $CO_3^{\bullet-}$ decay was, indeed, due to reaction 8 and was not a result of $CO_3^{\bullet-}$ scavenging by contaminants, such as HO_2^-, introduced with $ONOO^-$. The lower line in Figure 2 shows the observed first-order rate constant of $CO_3^{\bullet-}$ decay measured repeatedly over a period of time, during which the initially added 1 mM $ONOO^-$ partially decayed; the concentration of $ONOO^-$ remaining in solution was determined spectrophotometrically prior to each kinetic measurement. Again, k_{obs} was linearly dependent on $[ONOO^-]$ with the corresponding value of $k_8 = (7.7 \pm 1.2) \times 10^6\ M^{-1}$

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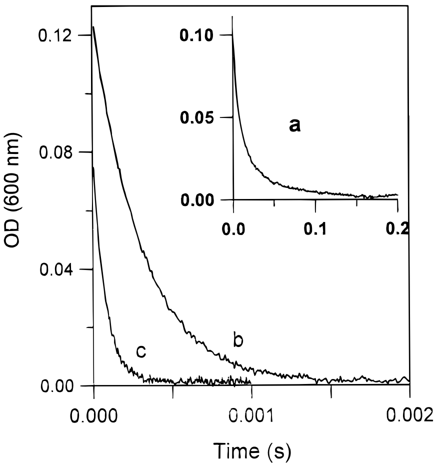

Figure 1. Kinetic traces of $CO_3^{\bullet-}$ radical decay monitored at 600 nm in the absence of peroxynitrite (a) and in the presence of 290 μM (b) and 1.3 mM peroxynitrite (c). All solutions were N_2O saturated and contained 0.2 M Na_2CO_3 at pH 12. The concentration of $CO_3^{\bullet-}$ at the end of the radiation pulse varied between 3.5 and 5.4 μM.

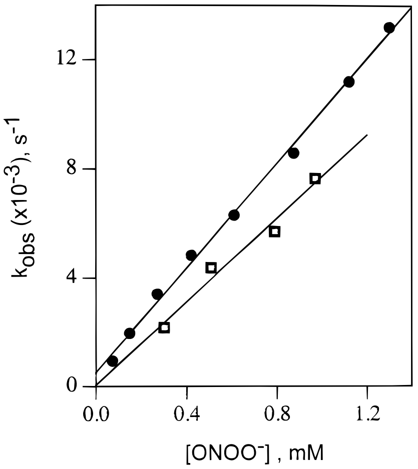

Figure 2. Observed first-order rate constant for the decay of $CO_3^{\bullet-}$ as a function of the peroxynitrite concentration. Variation of $[ONOO^-]$ by (●) diluting the stock peroxynitrite solution, $[CO_3^{\bullet-}]_o \sim 9$ μM, and (□) letting 0.97 mM $ONOO^-$ decay (details in the text), $[CO_3^{\bullet-}]_o \sim 3$ μM. All solutions were N_2O saturated and contained 0.2 M Na_2CO_3 at pH 12.

s^{-1}, somewhat lower than the value determined from the upper line in Figure 2.

A small intercept is expected for both lines in Figure 2 due to the contribution from the bimolecular self-decay of $CO_3^{\bullet-}$ and some scavenging by contaminating NO_2^-. However, if reactive impurities are present, a larger intercept is expected for the lower line. The lack of measurable intercepts indicates that there is no significant contribution from contaminants to the decay of $CO_3^{\bullet-}$. The slope of the lower line in Figure 2 should not be affected by the impurities (provided they do not decay simultaneously with $ONOO^-$), whereas reactive impurities should result in a larger slope of the upper line. Therefore, the value obtained from the lower curve in Figure 2 seems to be more reliable. The rate constant for reaction 8 at zero ionic strength $k_8 = (3.7 \pm 0.6) \times 10^6$ M^{-1} s^{-1} was calculated from the Debye−Huckel−Bronsted−Davies expression for the primary kinetic salt effect: $\log(k/k_o) = 1.02 Z_A Z_B I^{1/2}/(1 + I^{1/2}) - 0.2I$.

Hydroxyl Radical. This radical reacts rapidly with NO_2^- ($k(OH+NO_2^-) = 1.1 \times 10^{10}$ M^{-1} s^{-1}; $k(O^- + NO_2^-) = 3.1 \times 10^8$ M^{-1} s^{-1})[19] and HO_2^- ($k(OH+HO_2) = 7.5 \times 10^9$ M^{-1} s^{-1}; $k(O^- + HO_2) = 4.0 \times 10^8$ M^{-1} s^{-1}),[19] but relatively slowly with H_2O_2 ($k(OH+H_2O_2) = 2.7 \times 10^7$ M^{-1} s^{-1})[19] and NO_3^- ($k(OH+NO_3^-) = 1.4 \times 10^8$ M^{-1} s^{-1}).[19] To minimize the effects of these impurities we used peroxynitrite preparation *B* where contamination by both residual H_2O_2 and NO_2^- was less than 1% of the $ONOO^-$ concentration (see Experimental Section).

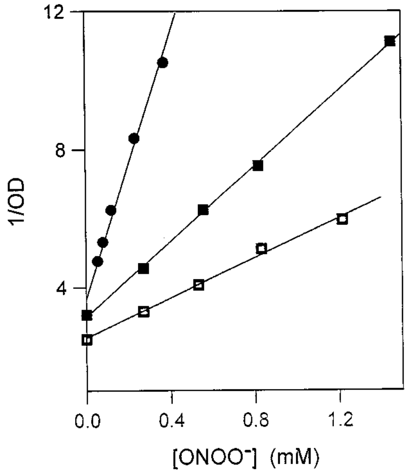

Figure 3. Reciprocal of the maximum absorption at 600 nm as a function of $[ONOO^-]$ in the presence of: (●) 3 mM CO_3^{2-} at pH 12, $[CO_3^{\bullet-}]_o = 15$ μM; (■) 10 mM CO_3^{2-} at pH 12, $[CO_3^{\bullet-}]_o = 15$ μM, and (□) 20 mM CO_3^{2-} at pH 12.6, $[CO_3^{\bullet-}]_o = 18$ μM. All solutions were N_2O saturated.

The rate constant for the reaction between $^{\bullet}OH/O^{\bullet-}$ and $ONOO^-$ was determined at pH 12 and 12.6 from competition between $ONOO^-$ and CO_3^{2-} (reactions 4 and 5) for the hydroxyl radical. If both $^{\bullet}OH$ and $O^{\bullet-}$ oxidize $ONOO^-$,

$$^{\bullet}OH + ONOO^- \rightarrow ONOO^{\bullet} + OH^- \tag{9}$$

$$O^{\bullet-} + ONOO^- \rightarrow ONOO^{\bullet} + OH^- \tag{10}$$

the effective rate constant is:

$$k_{11} = \frac{k_9[H^+]}{K_{OH} + [H^+]} + \frac{k_{10}K_{OH}}{K_{OH} + [H^+]} \tag{11}$$

The yield of $CO_3^{\bullet-}$ observed following pulse irradiation decreased with increasing the concentration of added $ONOO^-$ as shown in Figure 3. Under our experimental conditions, the half-life of the $CO_3^{\bullet-}$ formation was shorter than that of the reaction between $CO_3^{\bullet-}$ and $ONOO^-$ ($k_6[CO_3^{2-}]_o \gg k_8[ONOO^-]_o$), therefore the latter reaction had no effect on the yield of $CO_3^{\bullet-}$. Considering competition for the hydroxyl radicals between CO_3^{2-} (reactions 4 and 5) and $ONOO^-$ (reactions 9 and 10) we obtain the following for the yield of $CO_3^{\bullet-}$:

$$[CO_3^{\bullet-}] = [CO_3^{-}]_o k_6 [CO_3^{2-}]/(k_6[CO_3^{2-}]_o + k_{11}[ONOO^-]_o) \tag{12}$$

or, after rearrangement:

$$\frac{1}{[CO_3^{\bullet-}]} = \frac{1}{[CO_3^{\bullet-}]_o} + \frac{k_{11}[ONOO^-]_o}{k_6[CO_3^{\bullet-}]_o[CO_3^{2-}]_o} \tag{13}$$

where $[CO_3^{\bullet-}]_o$ is the yield of $CO_3^{\bullet-}$ in the absence of $ONOO^-$.

Equation 13 predicts a linear plot of $1/OD_{600}$ vs $[ONOO^-]_o$, as observed (Figure 3), with the slope-to-intercept ratio $S/I =$

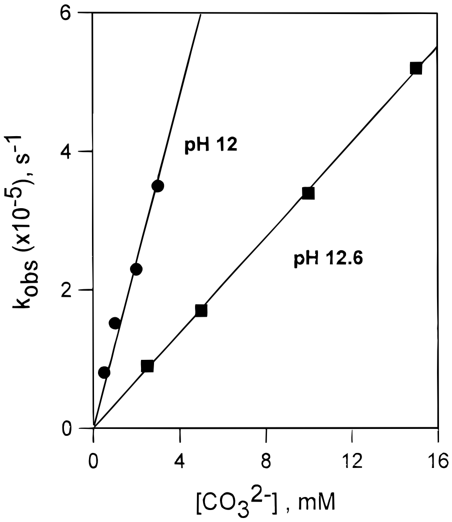

Figure 4. Dependence of the observed first-order rate constant for the formation of $CO_3^{\bullet -}$ on the carbonate concentration at pH 12 (●) and 12.6 (■). All solutions were N_2O saturated. The maximum concentrations of $CO_3^{\bullet -}$ varied between 3 and 11 μM.

$k_{11}/k_6[CO_3^{2-}]_o$. From data in Figure 3 for pH 12 we obtained $k_{11}/k_6 = 15.8$ and 17.3 in the presence of 3 and 10 mM carbonate, respectively. The latter value appears to be more reliable, because the loss of hydroxyl radicals due to their recombination can be neglected in more concentrated carbonate. In 20 mM carbonate at pH 12.6 the ratio $k_{11}/k_6 = 22$ was calculated from the data in Figure 3. Values of $k_6 = (1.2 \pm 0.1) \times 10^8$ and $(3.4 \pm 0.3) \times 10^7$ M^{-1} s^{-1} at pH 12 and 12.6, respectively, were determined in a separate experiment by following the rate of the formation of $CO_3^{\bullet -}$ as a function of $[CO_3^{2-}]$ at low doses (Figure 4). Using these values we obtained $k_{11} = (2.1 \pm 0.2) \times 10^9$ and $(7.5 \pm 0.7) \times 10^8$ M^{-1} s^{-1} at pH 12 and 12.6, respectively. From these rate constants and eq 11 we calculated $k_9 = (4.8 \pm 0.8) \times 10^9$ M^{-1} s^{-1} and $k_{10} < 1.7 \times 10^8$ M^{-1} s^{-1}.

Nitrogen Dioxide. Approximately 20−23 μM of $^{\bullet}NO_2$ radicals were generated by pulse irradiation of an Ar-saturated solution containing 0.46 M NO_3^- and 0.05 M NO_2^- at pH 12, where all the primary radicals formed in reaction 1 were converted into $^{\bullet}NO_2$ via reactions 2 and 14−16:

$$e^-_{aq} + NO_3^- \rightarrow NO_3^{2-} \qquad k_{14} = 9.7 \times 10^9\ M^{-1}\ s^{-1}\ (\text{ref } 19) \quad (14)$$

$$NO_3^{2-} + H_2O \rightarrow {}^{\bullet}NO_2 + 2OH^- \qquad k_{15} = 5.5 \times 10^4\ M^{-1}\ s^{-1}\ (\text{ref } 20) \quad (15)$$

$$^{\bullet}OH/O^{\bullet -} + NO_2^- \rightarrow {}^{\bullet}NO_2 + OH^- \qquad k_{16} = 6.4 \times 10^9\ M^{-1}\ s^{-1}\ (\text{pH } 12)\ (\text{ref } 19) \quad (16)$$

In the absence of added ONOO$^-$, the decay kinetics of $^{\bullet}NO_2$ monitored at 400 nm corresponded to the well-established pathway (reactions 17 and 18) with a measured half-life of 2.7 ms.

$$^{\bullet}NO_2 + {}^{\bullet}NO_2 \rightleftharpoons N_2O_4 \qquad k_{17} = 4.5 \times 10^8\ M^{-1}\ s^{-1};\ k_{-17} = 6.9 \times 10^3\ s^{-1}\ (\text{ref } 20) \quad (17)$$

$$N_2O_4 + H_2O \rightarrow NO_3^- + NO_2^- + 2H^+ \qquad k_{18} = 1 \times 10^3\ s^{-1}\ (\text{ref } 20) \quad (18)$$

This half-life was not shortened by the addition of up to 2.1 mM peroxynitrite. Taking into account that less than 20% acceleration of $^{\bullet}NO_2$ decay would be readily detectable, we can estimate an upper limit for the rate constant of the reaction of $^{\bullet}NO_2$ with ONOO$^-$ as 2.5×10^4 M^{-1} s^{-1}. This result is in quantitative agreement with the observation by Lymar and Hurst[10] that the decomposition of ONOO$^-$ is not accelerated when the $^{\bullet}NO_2$ radical is produced in the ONOO$^-$ − CO_2 − NO_2^- system. Based on kinetic simulation of their data, a very similar upper limit of 2×10^4 M^{-1} s^{-1} can be set for the rate constant of the reaction between $^{\bullet}NO_2$ and ONOO$^-$.

Azide Radical. We used peroxynitrite preparation *B* (see Experimental Section) to study the oxidation of ONOO$^-$ by this radical, because minimization of both residual H_2O_2 and NO_2^- may be equally important in this case. The azide radical reacts rapidly with HO_2^- ($k = 3.2 \times 10^9$ M^{-1} s^{-1}).[13] The rate constant for oxidation of NO_2^- by $^{\bullet}N_3$ has not been reported,[13] but it is not expected to be larger than that for oxidation of ClO_2^-, which is ca. 2×10^9 M^{-1} s^{-1}.[9]

When an N_2O-saturated solution containing 20 mM NaN_3 at pH 12 is irradiated, $^{\bullet}N_3$ is formed via reactions 1−3 and 19:

$$^{\bullet}OH/O^{\bullet -} + N_3^- \rightarrow {}^{\bullet}N_3 + OH^- \qquad k_{19} = 5.4 \times 10^9\ M^{-1}\ s^{-1}\ (\text{pH } 12)\ (\text{ref } 19) \quad (19)$$

About 3 μM $^{\bullet}N_3$ was produced by an electron pulse. The decay of $^{\bullet}N_3$ followed at 275 nm was second order with $2k = (9.8 \pm 1.0) \times 10^9$ M^{-1} s^{-1}, in agreement with the literature values.[13] The second-order kinetics turned into first order upon addition of 17−80 μM ONOO$^-$. The observed first-order rate constant was linearly dependent on the concentration of ONOO$^-$ (Table 1), yielding the rate constant $k_{20} = (7.2 \pm 0.9) \times 10^8$ M^{-1} s^{-1} for the reaction

$$^{\bullet}N_3 + ONOO^- \rightarrow ONOO^{\bullet} + N_3^- \quad (20)$$

This value of k_{20} was, within experimental error, identical to the value obtained from the kinetics of ONOO$^-$ bleaching observed at 302 nm.

Chlorine Dioxide. Preparation *B* of peroxynitrite (see Experimental) was used to study the oxidation of ONOO$^-$ by this radical. Peroxynitrite was relatively stable in the presence of $NaClO_2$ at pH 12; a loss of about 8% and 14% of ONOO$^-$ was detected within 30 min upon addition of 8 and 40 mM chlorite, respectively. The average time of each experiment was, therefore, kept under 10 min.

The $ClO_2^{\bullet}$ radical was generated by the pulse irradiation of N_2O-saturated solutions containing 24 mM $NaClO_4$ at pH 12 through reactions 1−3 and 21:

$$^{\bullet}OH/O^{\bullet -} + ClO_2^- \rightarrow ClO_2^{\bullet} + OH^- \qquad k_{21} = 3.0 \times 10^9\ M^{-1}\ s^{-1}\ (\text{pH } 12)\ (\text{ref } 19) \quad (21)$$

The formation and decay of $ClO_2^{\bullet}$ was followed at 420 nm, where ONOO$^-$ does not absorb. In the presence of ONOO$^-$, the $ClO_2^{\bullet}$ radical, which was otherwise stable, decayed exponentially, apparently in the reaction

$$ClO_2^{\bullet} + ONOO^- \rightarrow ONOO^{\bullet} + ClO_2^- \quad (22)$$

Table 1. The Observed First-Order Rate Constants for the Reactions of $^{\bullet}N_3$ and $ClO_2^{\bullet}$ with $ONOO^-$

	k_{obs}, s^{-1}	
$[ONOO^-]$, M	azide radical[a]	chlorine dioxide[b]
1.7×10^{-5}	$(3.0 \pm 0.3) \times 10^4$	
3.7×10^{-5}	$(4.7 \pm 0.3) \times 10^4$	
6.4×10^{-5}	$(5.8 \pm 0.5) \times 10^4$	
8.0×10^{-5}	$(7.9 \pm 0.4) \times 10^4$	
1.2×10^{-4}		3.0 ± 0.3
3.6×10^{-4}		11.8 ± 1.8
7.1×10^{-4}		22.5 ± 0.4
1.2×10^{-3}		33.9 ± 1.2
1.9×10^{-3}		64.5 ± 1.5

[a] Measured from the decays of $^{\bullet}N_3$ and $ONOO^-$ followed at 275 and 302 nm, respectively, in pulse-irradiated (dose 5 Gy) N_2O-saturated solutions containing 20 mM azide at pH 12. [b] Measured from the decay of $ClO_2^{\bullet}$. followed at 420 nm in pulse-irradiated (dose 26 Gy) N_2O-saturated solutions containing 24 mM chlorite at pH 12.

Table 2. Rate Constants and Redox Potentials of Various Radicals with $ONOO^-$ and HO_2^-

$R^{\bullet}/R^-$	$E°(R^{\bullet}/R^-)$ (V)	$k(R^{\bullet}/R^-)$[a] ($M^{-1}\ s^{-1}$)	$k(R^{\bullet}+ONOO^-)$ ($M^{-1}\ s^{-1}$)	$k(R^{\bullet} + HO_2^-)$ ($M^{-1}\ s^{-1}$)
$^{\bullet}OH/OH^-$	1.9[21]	300[23]	4.8×10^9	7.0×10^9 [13]
$CO_3^{\bullet -}/CO_3^{2-}$	1.59[22]	0.4[23]	3.7×10^6 ($I = 0$)	$(1.0-5.6) \times 10^7$ [13]
$^{\bullet}N_3/N_3^-$	1.33[21]	3.7×10^6 [24]	7.2×10^8	3.2×10^9 [13]
$^{\bullet}NO_2/NO_2^-$	1.04[21]	9.6[24]	$<2 \times 10^4$	n.d.
$ClO_2^{\bullet}/ClO_2^-$	0.934[21]	3.3×10^4 [24]	3.2×10^4	8.0×10^4 [13]
$HO_2^{\bullet}/HO_2^-$	0.75[21]	17[25]		

[a] The self-exchange rate constants have been calculated in the cited references by applying the Marcus cross relationship to the reactions between the corresponding species and a series of coordination compounds.

From the dependence of k_{obs} on the concentration of $ONOO^-$ given in Table 1, the rate constant $k_{22} = (3.2 \pm 0.3) \times 10^4$ $M^{-1}\ s^{-1}$ was determined.

Oxidation Potential of $ONOO^-$. Rate constants for the oxidation of $ONOO^-$ by the radicals determined in this study, the corresponding literature values for the oxidation of HO_2^-, and the redox potentials of the radicals are summarized in Table 2. It is apparent that these rate constants do not reflect the trend in the reduction potentials of the radicals. For instance, the rate constants for oxidation of both $ONOO^-$ and HO_2^- by $^{\bullet}N_3$ are much larger than those for oxidation by $CO_3^{\bullet -}$, which is a stronger oxidant. A similar effect was observed for the oxidation of many other substrates by these radicals,[13] which, most probably, reflects a much higher self-exchange rate for the $^{\bullet}N_3/N_3^-$ couple (Table 2). Although the reduction potential of $ClO_2^{\bullet}$ is somewhat lower than that of $^{\bullet}NO_2$, the rate constants for the one-electron oxidation by $ClO_2^{\bullet}$ are generally larger than those by $^{\bullet}NO_2$, also due to the higher self-exchange rate for the $ClO_2^{\bullet}/ClO_2^-$ couple (Table 2). On the basis of this trend, one expects that the reactivity of $ClO_2^{\bullet}$ toward $ONOO^-$ will exceed that of $^{\bullet}NO_2$, in agreement with our results.

Assuming that reaction 22 represents a simple one-electron-transfer we can estimate the oxidation potential of $ONOO^-$ using Marcus formalism by comparing the rate constant for $ONOO^-$ oxidation obtained here with the corresponding value for the similar reaction of HO_2^- (Table 2). From the Marcus equation $k_{12} = (k_{11}k_{22}K_{12}f_{12})^{1/2}$ (here k_{12} is the electron-transfer rate constant for the cross reaction, k_{11} and k_{22} are the self-exchange rate constants for the reactants, K_{12} is the cross reaction equilibrium constant, and $\ln f_{12} = (\ln K_{12})^2/4 \ln(k_{11}k_{22}/10^{22})$),[26] the following ratios were derived:

$$\frac{k_{12}{}^2(HO_2-/ClO_2^{\bullet})}{k_{12}{}^2(ONOO^-/ClO_2^{\bullet})} = C\frac{K_{12}(HO_2^-/ClO_2^{\bullet})}{K_{12}(ONOO^-/ClO_2^{\bullet})} \qquad (23)$$

$$C = \frac{k_{11}(HO_2^{\bullet}/HO_2^-)}{k_{11}(ONOO^{\bullet}/ONOO^-)}\,\frac{f_{12}(HO_2^-/ClO_2^{\bullet})}{f_{12}(ONOO^-/ClO_2^{\bullet})} \qquad (24)$$

When the reaction driving force is not very large, factor f_{12} is close to unity, e.g., $f_{12} = 0.71$ for the oxidation of HO_2^- by $ClO_2^{\bullet}$. If the magnitudes of k_{11} and K_{12} are not vastly different for HO_2^- and $ONOO^-$, factor C should also be close to unity. With this assumption, we calculated $E°(ONOO^{\bullet}/ONOO^-) = 0.8$ V. Note that this result is not very sensitive to the value of C chosen; variation of C between 0.1 and 10 changes $E°(ONOO^{\bullet}/ONOO^-)$ from 0.86 to 0.74 V. From the corresponding data in Table 2 for the $^{\bullet}N_3$ radical, we obtained $E°(ONOO^{\bullet}/ONOO^-) = 0.83$ V, close to the former estimate. Both estimates are also in good agreement with the thermodynamic estimate of 0.9 V.[12]

Implications for the Peroxynitrite Decomposition. In the presence of CO_2, the decomposition of peroxynitrite yields 30–35% of $CO_3^{\bullet -}$ and $^{\bullet}NO_2$.[8–10] Because the rate constants for oxidation of $ONOO^-$ by both $CO_3^{\bullet -}$ and $^{\bullet}NO_2$ are rather small (Table 2), the contribution from these reactions to the overall decomposition is predicted to be minor even under the most favorable catalytic conditions, i.e., when $[ONOO^-] > [CO_2]$. This conclusion is supported by numerical simulations of the CO_2-catalyzed peroxynitrite decomposition kinetics, which we performed using previously reported data[7–10] and the rate constants from Table 2.

A strong case was recently made by Merenyi and Lind[12d] that the decomposition of ONOOH proceeds via the homolytic cleavage of its peroxy bond producing a geminate pair of the $^{\bullet}OH$ and $^{\bullet}NO_2$ radicals. It was concluded that the observed ca. 40% yield of the indirect oxidation of various substrates by peroxynitrite[5] represents the cage escape probability for the geminate pair of the $^{\bullet}OH$ and $^{\bullet}NO_2$ radicals. The large rate constant for reaction 9 determined in this study suggests that, in the absence of other reactants, $^{\bullet}OH$ will be efficiently scavenged by $ONOO^-$, yielding $ONOO^{\bullet}$ even at pH as low as 5. The fate of the $ONOO^{\bullet}$ is unexplored. By analogy with the $HO_2^{\bullet}$ radical, $ONOO^{\bullet}$ may disproportionate yielding oxygen (reaction 25) with subsequent hydrolysis of $^{\bullet}NO_2$ (reactions 17 and 18).

$$ONOO^{\bullet} + ONOO^{\bullet} \rightarrow 2\ ^{\bullet}NO_2 + O_2 \qquad (25)$$

Alternatively, the $ONOO^{\bullet}$ radical may undergo decomposition (reaction 26) followed by reactions 27 and 28.

$$ONOO^{\bullet} \rightleftharpoons {}^{\bullet}NO + O_2 \qquad (26)$$

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$$^{\bullet}NO_2 + {}^{\bullet}NO \rightleftharpoons N_2O_3 \qquad k_{27} = 1.1 \times 10^9\ M^{-1}\ s^{-1};\ k_{-27} = 8.4 \times 10^4\ s^{-1}\ \text{(ref 27)} \tag{27}$$

$$N_2O_3 + H_2O \rightarrow 2NO_2^- + 2H^+ \qquad k_{28} = (2 \times 10^3) + (1 \times 10^8)[OH^-]\ s^{-1}\ \text{(ref 28)} \tag{28}$$

Indeed, ab initio calculations by McKee[12a] suggest that the $ONOO^{\bullet}$ radical is unstable by about 11.5 kcal/mol with respect to reaction 26. In addition, it was recently reported that molecular oxygen is found among the products of peroxynitrite decomposition at pH 6−9.[6,10] Concentrations of $ONOO^-$ used in these studies were high enough to make reaction 9 an important pathway for the $^{\bullet}OH$ radical decay. It is therefore possible that reactions 25 and 26 are, at least in part, responsible for the observed O_2 generation.[27,28]

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