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# Product Distribution of Peroxynitrite Decay as a Function of pH, Temperature, and Concentration

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***Abstract:*** The decay of peroxynitrite [O=NOO$^-$, oxoperoxonitrate(1$-$)] was examined as a function of concentration (0.050$-$2.5 mM), temperature (5$-$45 °C), and pH (2.2$-$10.0). Below 5 °C and pH 7, little amounts of the decomposition products nitrite and dioxygen are formed, even when the peroxynitrite concentration is high (2.5 mM). Instead, approximately $\geq$90% isomerizes to nitrate. At higher pH, decomposition increases at the expense of isomerization, up to nearly 80% at pH 10.0 at 5 °C and 90% at 45 °C. Much less nitrite and dioxygen per peroxynitrite are formed when the peroxynitrite concentration is lower; at 50 $\mu$M and pH 10.2, $\leq$40% decomposes. In contrast to two other reports (Pfeiffer, S.; Gorren, A. C. F.; Schmidt, K.; Werner, E. R.; Hansert, B.; Bohle, D. S.; Mayer, B. *J. Biol. Chem.* **1997**, *272*, 3465$-$3470, and Coddington, J. W.; Hurst, J. K.; Lymar, S. V. *J. Am. Chem. Soc.* **1999**, *121*, 2438$-$2443), we find that the extent of decomposition is dependent on the peroxynitrite concentration.

## Introduction

At a concentration in the nanomolar range, nitrogen monoxide acts as a messenger that is involved in, for instance, the relaxation of blood vessels. Much higher concentrations, in the micromolar range, of both nitrogen monoxide and superoxide are produced by activated macrophages during the immune response. Under these conditions, peroxynitrite may be formed from the very fast reaction between superoxide and nitrogen monoxide.[1-3] Because peroxynitrous acid (p$K_a$ = 6.8) is an oxidant and a nitrating agent, it is harmful to membranes, proteins, and nucleic acids. In the absence of molecules to react with, peroxynitrous acid isomerizes to nitrate at a rate of 1.2 s$^{-1}$ at 25 °C. As many of the oxidations and nitrations are zero-order in the molecules that are modified and first-order in peroxynitrous acid, it is widely assumed that peroxynitrous acid undergoes homolysis to the hydroxyl radical and nitrogen dioxide. In addition to isomerization, at neutral to alkaline pH decomposition[4,5] to dioxygen and nitrite also takes place.[6] The ratio of these two processes appears to be dependent on pH and temperature.[7]

We have presented kinetic evidence for an adduct between the peroxynitrite anion and peroxynitrous acid; deviations from the first-order decay of peroxynitrous acid were observed at and above the p$K_a$ of peroxynitrous acid, where both species are present, and at total concentrations of peroxynitrite of 0.1 mM or more.[3] These deviations were clearly of second-order in total peroxynitrite concentration. We suggested that the peroxynitrite anion and peroxynitrous acid form an adduct that reacts to form dioxygen and nitrite, eq 1. This mechanism implies that the

$$\mathrm{ONOOH} + \mathrm{ONOO}^- \rightarrow (\mathrm{ONOOHONOO})^- \rightarrow 2\,\mathrm{NO_2}^- + \mathrm{O_2} + \mathrm{H}^+ \qquad (1)$$

disproportionation to nitrite and dioxygen depends on the total concentration of peroxynitrite, whereas the isomerization to nitrate is strictly monomolecular. Pfeiffer et al.[7] claim that the formation of nitrite and dioxygen is not dependent on the total peroxynitrite concentration; this is reflected in their proposed mechanism, which involves homolysis of the O$-$O bond of ONOOH, with subsequent reactions of the products to arrive at dioxygen and nitrite. A related mechanism, invoking an additional homolysis of ONOO$^-$ to yield superoxide and

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(4) Koppenol, W. H. In *Interrelations between Free Radicals and Metal Ions in Life Processes*; Sigel, A., Sigel, H., Eds.; Marcel Dekker: New York, 1999; pp 597$-$619. The disappearance of peroxynitrite by any process will be referred to as decay; the process that yields nitrate is named isomerization, and that which results in nitrite and dioxygen is named decomposition, in agreement with IUPAC conventions.[5] The decomposition of peroxynitrite can also be regarded as a disproportionation reaction, according to the set of reactions below. These reactions only serve to illustrate the concept; we do not imply that they actually take place.

$$\begin{gathered}
\mathrm{ONOO}^- \rightarrow \mathrm{ONOO}^\bullet + \mathrm{e}^- \\
\mathrm{ONOOH} + \mathrm{H}^+ + \mathrm{e}^- \rightarrow \mathrm{NO_2}^\bullet + \mathrm{H_2O} \\
\mathrm{ONOO}^\bullet \rightarrow \mathrm{NO}^\bullet + \mathrm{O_2} \\
\mathrm{NO_2}^\bullet + \mathrm{NO}^\bullet \rightarrow \mathrm{N_2O_3} \\
\mathrm{N_2O_3} + \mathrm{H_2O} \rightarrow 2\mathrm{NO_2}^- + 2\mathrm{H}^+ \\
\hline
\mathrm{ONOOH} + \mathrm{ONOO}^- \rightarrow 2\mathrm{NO_2}^- + \mathrm{O_2} + \mathrm{H}^+
\end{gathered}$$

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nitrogen monoxide and reactions between the products of the two homolyses, has been proposed by Hurst and co-workers.[8,9] We show here that the amounts of dioxygen and nitrite formed, relative to the initial concentration of peroxynitrite, depend on the initial concentration of peroxynitrite. A bimolecular reaction of peroxynitrite with peroxynitrous acid, as established for other peracids, provides the simplest explanation of the observed product distribution.

## Experimental Section

**Chemicals.** Nitrogen monoxide was obtained from Linde, and argon (99.998%) was obtained from Pangas ($O_2 < 2$ ppm). All other chemicals, except peroxynitrite, were purchased from Fluka and Merck and were at least analytical grade, except for potassium superoxide, which was synthetic grade. HPLC grade water was produced from deionized water by means of a Millipore water purification unit. Peroxynitrite in alkaline solution (pH 12) was prepared from solid potassium superoxide and nitrogen monoxide as described previously[10] and contained 10−20% nitrite as the only significant impurity. It was also tested for residual hydrogen peroxide with the titanyl reagent after elimination of peroxynitrite by acidification to pH 2. The detection limit for hydrogen peroxide by titanyl is 5 $\mu$M.[11] Since hydrogen peroxide reacts with peroxynitrous acid[12] and with nitrous acid to produce peroxynitrous acid,[13,14] it is important to remove it. Pure tetramethylammonium peroxynitrite was synthesized according to Bohle et al.[15]

**Nitrate and Nitrite Determinations.** Experiments on the decay of peroxynitrite were carried out in a borosilicate glass vessel immersed in a Lauda bath of constant temperature. Peroxynitrite solutions were kept in an ice bath at 0 °C. Phosphate buffer solutions were kept in another Lauda bath at a higher temperature, such that the temperature upon mixing with peroxynitrite matched that of the reaction vessel temperature. Two peroxynitrite concentrations were used, 5 mM and 100 $\mu$M, both in 5 mM sodium hydroxide, and the concentrations of the phosphate buffers were 0.2 M, resulting in final concentrations of 2.5 mM or 50 $\mu$M peroxynitrite and 0.1 M phoshate buffer. Both solutions were saturated with argon during thermal conditioning. Care was taken to avoid any contact between solutions and metal surfaces. The preconditioned solutions were mixed rapidly in the thermostated vessel by means of two equal polypropylene syringes with Teflon tubing attached. The reaction was allowed to proceed for at least seven half-lives of the isomerization, calculated from the activation energy of 85 kJ/mol,[16] for the given pH and temperature. To avoid disproportionation of nitrous acid, solutions of pH below 6 were brought to pH $\approx 7$ by addition of sodium hydroxide after the reaction was finished. A dilute solution of each peroxynitrite stock solution (around 500 $\mu$M) was left to isomerize at 0 °C and pH 3. The solution then was brought quickly to pH $\approx 7$ by addition of appropriate amounts of sodium hydroxide solution and analyzed for nitrite and nitrate. The nitrate concentration was compared with the peroxynitrite concentration measured spectrophotometrically in the stock solution at 302 nm ($\epsilon = 1705$ M$^{-1}$ cm$^{-1}$)[15] and was found to be identical within ±3% for three determinations. The nitrite concentration obtained from this determination was used later to correct the nitrite concentration found in the decomposition experiments. Nitrate and nitrite were determined by ion chromatography with a Hamilton PRP X-100 anion exchanging column (125 × 4 mm) attached to a Hewlett-Packard HPLC apparatus. A phthalate solution (pH 8.6, 2 mL/min) was used as eluent. Anions were detected and quantified by measuring the absorbance decrease at 360 nm caused by anion displacement of phthalate in the eluent. The accuracy of nitrate and nitrite measurements was ±5% as determined with standard solutions of sodium nitrate and nitrite of concentrations from 1 to 100 $\mu$M. Analytes were diluted to that concentration range when necessary. At every pH, temperature, and concentration, three samples were analyzed, which results in a final error of $(5/\sqrt{3})$% or 3%. The pH was measured directly after the decomposition reaction was finished. At a later time, when such equipment became available, nitrite and nitrate were also determined by ion chromatography (Hamilton PRP X-100, 125 × 4 mm, 2 mL/min) with a phthalate eluent of pH 5 and conductometric detection; this method is more sensitive and has a wider linear range. Furthermore, nitrite was determined by the Griess method and by differential pulse polarography as *N*-nitrosodiphenylamine.[17]

**Dioxygen.** To determine dioxygen, the decay experiments were carried out in a two-necked flask with two Teflon tubing inlets mounted for introduction of the reactant solutions and a Teflon tubing outlet for pressure equilibration. The outlet was immersed in a vessel filled with phosphate buffer, which acted as a gas lock. All tubing and the dioxygen sensor were installed through gastight rubber septum seals. A magnetic stirring bar was placed inside to accelerate mixing. The total volume, 80 mL, was determined by the weight difference between the fully equipped flask completely filled with water and the same filled with air. After the flask had been filled with argon, 10 mL of peroxynitrite solution (5 mM, 2.5 mM, 0.8 mM, 0.4 mM, or 0.2 mM in 5 mM sodium hydroxide) and 10 mL of phosphate buffer (0.2 M, pH 8.5) were injected simultaneously, which left 60 mL occupied by gas. The sensor, a Clark cell with a separate electrolyte chamber and a gas-permeable membrane window (Ingold), was mounted in the gas space above the liquid. This position is important for optimum detection; due to the low solubility in aqueous electrolyte solutions, most of the dioxygen produced is transferred to the gas phase. Given a volume of 20 mL of aqueous solution and 60 mL of gas, the ratio of moles of dioxygen in the gas phase versus that in the liquid phase is ca. 90:1 at 25 °C. The positioning of the Clark cell in the gas phase also prevented contact between its steel housing and the solutions, which is an additional advantage. Disadvantages of this experimental setup are a delay between dioxygen evolution and detection, caused by the slow diffusion of dioxygen across the membrane into the electrolyte chamber of the Clark cell. The polarographic process of the detection is based on the consumption of some dioxygen with the consequence that, at very low dioxygen pressures, the membrane diffusion cannot match the consumption rate inside the Clark cell, which results in an underestimation of the partial dioxygen pressure. The Clark cell was connected to a potentiostat/amplifier from Ingold. The amplifier output was recorded versus time by a computer equipped with an analogue to digital converter board. The signal range of the Clark cell was calibrated with argon and atmospheric dioxygen. The pressure increase caused during injection was allowed to vent through the outlet; all openings were then sealed, and the change in dioxygen partial pressure was monitored. After completion, the pH was measured with a glass electrode (Ingold). The final value varied between 8.9 and 9.8. Each experiment was carried out three times. The system was also checked for atmospheric leaks by injection of argon-saturated solutions of sodium hydroxide and phosphate buffer, followed by subsequent monitoring of the dioxygen partial pressure for 1 h.

Errors are given as the standard deviation multiplied by 2.

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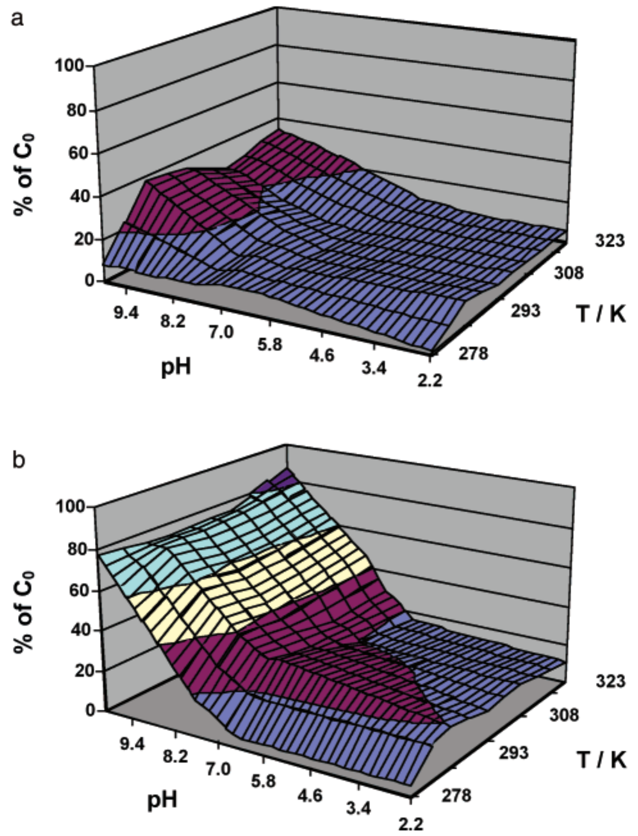


***Figure 1.*** Relative yield of nitrite from peroxynitrite and peroxynitrous acid at an ionic strength of 0.10 M as a function of temperature and pH. (a) $c_0 = 50\ \mu$M; (b) $c_0 = 2.5$ mM. A table with the results of the analytical determinations used to construct this figure is given under Supporting Information.

## Results

**Temperature, pH, and Concentration.** Figure 1a, for 50 $\mu$M peroxynitrite, and 1b, for 2.5 mM peroxynitrite, show relative nitrite yields as a function of pH (2.2−10.0) and temperature (5−45 °C). Below 5 °C, at low pH and 50 $\mu$M peroxynitrite, nitrate is formed with a yield of more than 90% (Figure 1a). Even a high concentration of 2.5 mM peroxynitrite yields at least 90% nitrate under these conditions of pH and temperature. Above 10 °C, the trends in Figure 1a and b are qualitatively similar: below pH 7 there is very little dependence on pH; at higher values, the yields of nitrite rises to ca. 30% and 80% at pH 10 for 50 $\mu$M and 2.5 mM peroxynitrite, respectively. The most pronounced difference between Figure 1a and b is due to the concentration of peroxynitrite. The undulating surface of the contour plots in Figure 1a and b should not be overinterpreted; minor variations are simply a consequence of fluctuations in the HPLC analysis (±3%). However, the upward trend at pH values above 7 and the trend downward at temperatures below 10 °C are significant. The results of the analytical determinations of nitrite and nitrate are given under Supporting Information. We found that there was an excellent mass balance; the concentrations of nitrite and nitrate as determined by ion chromatography added up to that of peroxynitrite within the analytical error.

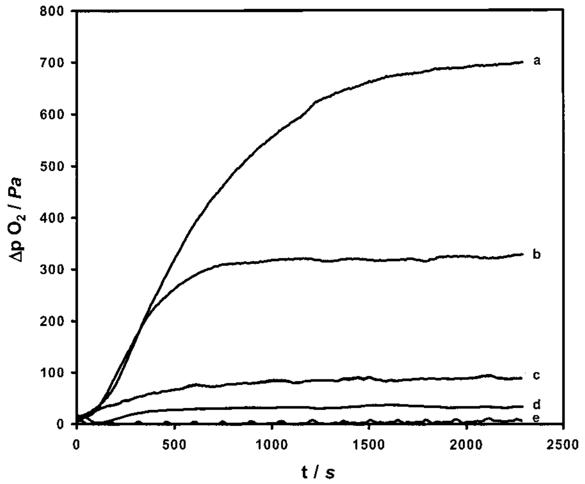


***Figure 2.*** Partial pressure of dioxygen during peroxynitrite decomposition at pH ≈ 9, ionic strength 0.1 M, and 22 °C. (a) $c_0 = 2.5$ mM; (b) $c_0 = 1.25$ mM; (c) $c_0 = 0.40$ mM; (d) $c_0 = 0.20$ mM; and (e) $c_0 = 0.10$ mM. The lag phase is caused by slow diffusion of dioxygen through the membrane window.

**Dioxygen.** According to eq 1, 1 equiv of dioxygen should be produced for 2 equiv of nitrite. To test this stoichiometry, the pH and the total peroxynitrite concentration were carefully selected to obtain amounts of dioxygen that could be well quantified. For this reason, experiments were carried out at pH ≈ 9 and room temperature (22 °C) with peroxynitrite concentrations of 2.5, 1.25, 0.40, 0.20, and 0.10 mM. The yield of dioxygen from 2.5 mM peroxynitrite can be compared directly to the corresponding nitrite experiment (Figure 1b); the other concentrations were selected to study the effect of dilution. Typical partial pressure versus time profiles are shown in Figure 2, and final dioxygen concentrations are shown in Table 1. The percentage of dioxygen at pH ≈ 9 is 70% for 2.5 mM peroxynitrite, which implies that 30% of the peroxynitrite was converted to nitrate. This result fits very well with the nitrite yield for 2.5 mM peroxynitrite at that pH. From the profiles in Figure 1a and b, it can be estimated that the conversion to nitrite and dioxygen may approach 100% at higher pH values and higher peroxynitrite concentrations. This is consistent with the observation that alkaline (pH > 10) stock solutions of peroxynitrite are converted mainly to nitrite upon aging and that dilute alkaline stock solutions are more stable. At 1.25 mM, the dioxygen yield percentage is slightly lower (62%), which demonstrates the bimolecular nature of the reaction that yields

***Table 1.*** Yields of Dioxygen from Peroxynitrite Decomposition at Room Temperature (pH 8.9−9.8)

| peroxynitrite concentration (mM) | $\Delta p$ O$_2$ (Pa) | moles O$_2$ in liquid phase[a] ($\mu$mol) | moles O$_2$ in gas phase[b] ($\mu$mol) | total moles O$_2$ ($\mu$mol) | calculated maximum yield[c] ($\mu$mol) | percentage of calculated maximum yield |
|---|---|---|---|---|---|---|
| 2.5 | 705 ± 10 | 0.2 | 17.4 | 17.6 | 25 | 70 |
| 1.25 | 310 ± 11 | 0.08 | 7.64 | 7.72 | 12.5 | 62 |
| 0.4 | 89 ± 10 | 0.01 | 2.09 | 2.10 | 4 | 53 |
| 0.2 | 45 ± 10 | 0.009 | 1.08 | 1.09 | 2 | 55 |
| 0.1 | 6 ± 8 | 0.000 | 0.14 | 0.14[d] | 1 | 14 |

[a] $k_H = 1.36 \times 10^{-8}$ M/Pa, volume 20 mL. [b] Volume 60 mL. [c] 20 mL of peroxynitrite solution. [d] Dioxygen partially consumed by Clark electrode.

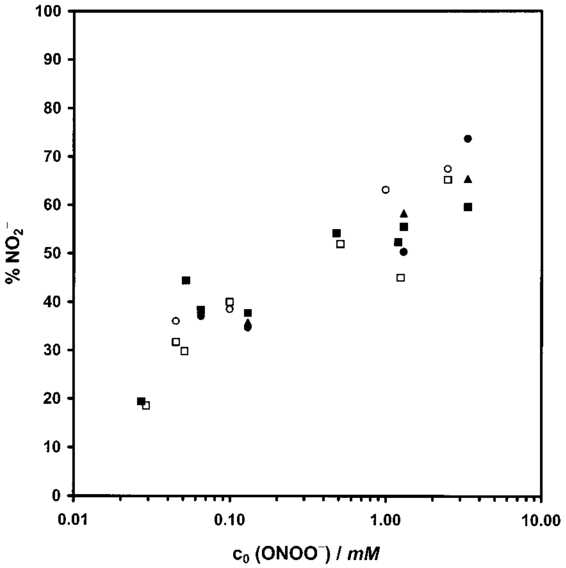


***Figure 3.*** Nitrite yields relative to initial concentrations of peroxynitrite (note logarithmic scale) corrected to pH 9. The final pH of the decomposed peroxynitrite solutions varied between pH 8.3 and 9.8, and the yields were corrected to pH 9.0 by linear interpolation of the data shown in Figure 1a and b. Open symbols: potassium peroxynitrite. Closed symbols: tetramethylammonium peroxynitrite. Triangles: Griess, spectrophotometric detection. Squares: anion exchange chromatography with conductometric detection. Circles: polarographic detection as *N*-nitrosodiphenylamine. Griess results with large systematic deviations are not shown.

***Table 2.*** Comparison of Nitrite and Dioxygen Yields

| peroxynitrite concentration[a] (mM) | nitrite yield ($\mu$mol) | dioxygen yield ($\mu$mol) | ratio |
|---|---|---|---|
| 2.5 | 36.5 | 17.6 | 2.1:1 |
| 0.4 | 4.4 | 2.10 | 2.1:1 |
| 0.2 | 2.10 | 1.09 | 1.9:1 |
| 0.1 | 0.72 | 0.14[b] | 5.1:1 |

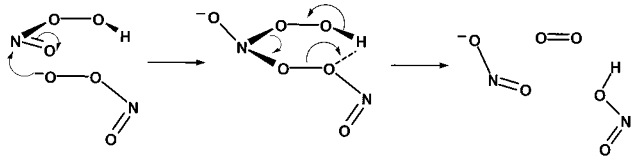
[a] Volume 20 mL. [b] Dioxygen partially consumed by Clark electrode.

nitrite and dioxygen, and the absolute amount of dioxygen found is close to one-half that obtained with 2.5 mM peroxynitrite. At even lower initial concentrations, the relative dioxygen yield is less, in line with the nitrite yields shown in Figure 1. Figure 3 shows the relative decomposition, that is the concentration of nitrite divided by the peroxynitrite concentration at the start, as a function of peroxynitrite concentration at pH 9. Nitrite was determined by ion chromatography, polarography, and by the Griess method, and nitrate was determined by ion chromatography. The percentages shown in Figure 3 were obtained by correcting experimentally determined yields. The mixing of alkaline peroxynitrite with the buffer resulted in pH values between 8.3 and 9.8; with the data from Supporting Information, the yields were corrected by linear interpolation to give percentages for pH 9. The ratio of nitrite to dioxygen yields is a constant 2:1 (Table 2), in agreement with other reports.[7−9] These results confirm the importance of decomposition relative to isomerization in peroxynitrite chemistry in alkaline media and of the bimolecular nature of the process.

## Discussion

While peroxynitrous acid isomerizes to nitrate, the peroxynitrite anion does not. For this reason, all nitrate found is believed to be (see below) the product of the isomerization of peroxynitrous acid, a reaction that is first-order in the acid.

***Scheme 1.*** The Peracid Mechanism as Applied to Peroxynitrous Acid



Decomposition of peroxynitrite is less well investigated, and several types of reaction must be considered:

$$2\ \mathrm{HOONO} \rightarrow 2\ \mathrm{HNO_2} + \mathrm{O_2} \tag{2}$$

$$\mathrm{HOONO} + \mathrm{ONOO^-} \rightarrow 2\ \mathrm{NO_2^-} + \mathrm{O_2} + \mathrm{H^+} \tag{3}$$

$$2\ \mathrm{ONOO^-} \rightarrow 2\ \mathrm{NO_2^-} + \mathrm{O_2} \tag{4}$$

Equation 2 should compete with the isomerization at low pH, whereas eq 3 is important at pH 6 and higher, since the p$K_a$ of peroxynitrous acid is 6.8 at an ionic strength of 0.1 M and 25 °C.[18] Equation 4 could compete with eq 3, especially at pH > 8. From the profiles in Figure 1a and b, we conclude that eq 2 can only marginally compete with isomerization, and eq 4 is surely slower than eq 3, otherwise it would lead to almost complete conversion to nitrite above pH 9, which is not the case. Thus, below pH 7, isomerization predominates and is independent of the total peroxynitrite concentration, while at pH 7 and higher *and* when the total peroxynitrite concentrations exceed 0.1 mM, the bimolecular decomposition (eq 3) becomes increasingly more important. Near pH 9, this is the major pathway of peroxynitrite decay, in agreement with our earlier finding that the decay kinetics of peroxynitrite show a second-order deviation under these conditions.[3] At low pH and temperatures below 10 °C, peroxynitrous acid is nearly quantitatively converted to nitrate, and these conditions are therefore employed for the determination of the background nitrite produced during the synthesis (see Experimental Section).

The results of the nitrate and nitrite determinations by ion chromatography showed good mass balance, with errors corresponding to the experimental limits of the HPLC determination (see also Supporting Information). This finding indicates that no gases, for example, nitrogen oxides, were formed. Furthermore, there is very good agreement between the nitrate and nitrite yields and the oxygen determinations (Table 2). The ratio of nitrite versus dioxygen of 2:1 and the increase in nitrite yield with pH found earlier[7,9] were confirmed. A concentration dependence of the nitrite and dioxygen yields on the total peroxynitrite concentration was revealed by covering a wider concentration range than in those works. We also showed that this is not a threshold phenomenon, but that the increase in nitrite and dioxygen per peroxynitrite is continuous with increasing peroxynitrite concentration. The mechanism proposed here for the decomposition of peroxynitrite is identical to that established for the peroxyacids peroxymonosulfuric acid [hydrogen trioxoperoxosulfate(1−)][19] and several peroxybenzoic acids.[20] The mechanism for decomposition, adapted for peroxynitrite, is given in Scheme 1. In contrast to peroxynitrous acid, these peracids

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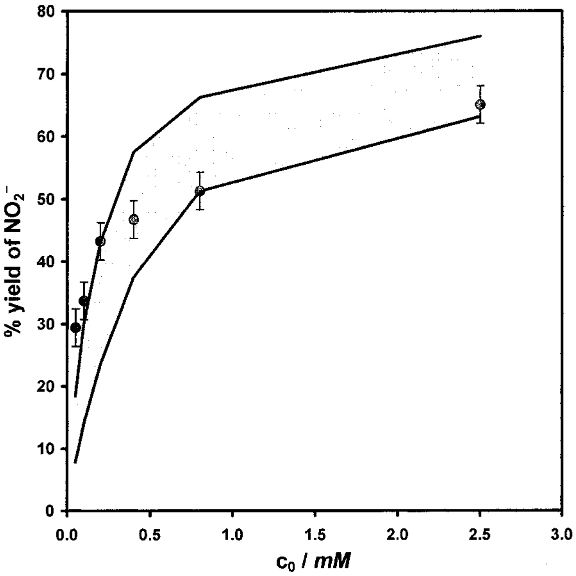

***Figure 4.*** Simulation of the decomposition to nitrite and dioxygen. Relative decomposition as a function of peroxynitrite concentration at pH 9 and 25 °C. The upper line is the result of a simulation (see text) for $k_8 = 0.3$ s$^{-1}$ and pH 9.4, and the lower line is for $k_8 = 0.2$ s$^{-1}$ and pH 8.7.

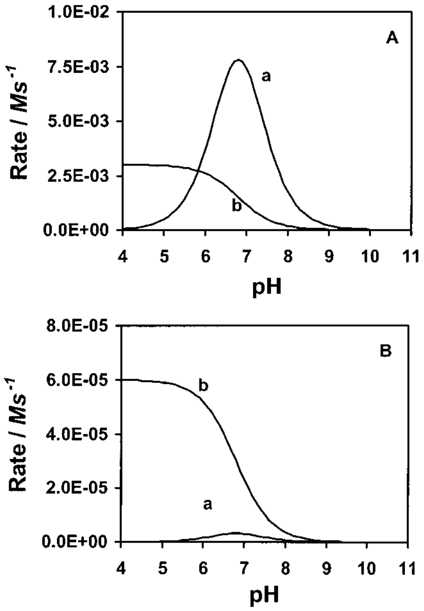

***Figure 5.*** Change of initial nitrate and nitrite formation rates with pH and peroxynitrite concentrations. At high peroxynitrite concentrations, decomposition is prevalent; however, while this process is taking place isomerization is becoming more important, until, as shown here for 50 $\mu$M, it is the predominant pathway. (A) [HOONO] + [ONOO$^-$] = 2.5 mM, (B) [HOONO] + [ONOO$^-$] = 50 $\mu$M; (a) nitrite production rate, and (b) nitrate production rate. $k_6 = 1.2$ s$^{-1}$, $k_7 = 2.5 \times 10^5$ M$^{-1}$ s$^{-1}$, $k_{-7} = 25$ s$^{-1}$, and $k_8 = 0.25$ s$^{-1}$ (between 0.2 and 0.3 s$^{-1}$ used in Figure 4).

are stable, and the rates of decomposition are maximal at the p$K_a$ of the peroxyacid.

The yield of nitrite has been modeled as a function of the peroxynitrite concentration at pH 9 and $T = 25$ °C and compared with experimental points from the anion exchange chromatography analysis shown in Figure 4. The reactions that were taken in account are

$$ONOO^- + H^+ \overset{K_a}{\rightleftharpoons} ONOOH \qquad (5)$$

$$ONOOH \rightarrow H^+ + NO_3^- \qquad (6)$$

$$ONOO^- + ONOOH \overset{K_{ADD}}{\rightleftharpoons} ADDUCT^- \qquad (7)$$

$$ADDUCT^- \rightarrow 2\,NO_2^- + O_2 + H^+ \qquad (8)$$

The parameters are $K_a = 10^{6.8}$, $k_6 = 1.2$ s$^{-1}$,[18] $k_7 = 2.5 \times 10^5$ M$^{-1}$ s$^{-1}$, with $k_{-7} = 25$ s$^{-1}$, such that $K_{ADD} \approx 10^4$ M$^{-1}$ as estimated before;[3] $k_8$ was varied between 0.2 and 0.3 s$^{-1}$ and the pH was varied between 8.7 and 9.4 to obtain a measure for interpolation uncertainties. The error bars on the experimental data represent only errors in the concentration measurements, not those introduced by the interpolation. The fit is reasonably good (Figure 4) and can even be improved by considering additional pathways for the decay of the adduct: isomerization to two nitrates or formation of nitrate, nitrogen dioxide, and the hydroxyl radical. The latter reaction is thermodynamically less favorable than isomerization, but differs little with respect to the energetics of the disproportionation reaction.[3] These alternative pathways are surely of secondary importance, but they would affect the curves in Figure 4.

A combination of decomposition (eq 3) and isomerization (eq 6) explains the peroxynitrite decay in the pH range 4 to 10. Below pH 7, the isomerization is faster than decomposition; around pH 7, decomposition begins to compete, because peroxynitrous acid and the anion are present in comparable concentrations. Decay would reach the maximum rate here were decomposition the only reaction to take place. However, at this pH the isomerization still produces more nitrate. While the isomerization rate

$$d[HOONO]/dt = -k_5[HOONO] \qquad (9)$$

decreases linearly with decreasing [H$^+$], the decomposition rate

$$d[HOONO]/dt = -k_{dec}[HOONO][ONOO^-] \qquad (10)$$

decreases more slowly because [ONOO$^-$] increases and compensates for the deficiency in [HOONO]. As a result, at peroxynitrite concentrations greater than 1 mM, nitrite production is favored above pH 7 and exceeds nitrate formation at pH > 9. Although initially decomposition may predominate in such a solution, isomerization becomes important after a certain amount of peroxynitrite has decayed. We have attempted to show this in Figure 5, which shows the *initial* contributions of isomerization and decomposition to the decay of a 2.5 mM and a 50 $\mu$M peroxynitrite solution at 25 °C as a function of pH with an estimated rate constant of $2 \times 10^4$ M$^{-1}$ s$^{-1}$ for the decomposition reaction; this rate constant may be refined.

Both Pfeiffer et al.[7] and Hurst and co-workers[8,9] proposed homolysis of peroxynitrous acid into nitrogen dioxide and the hydroxyl radical, and subsequent reactions, to arrive at nitrite and dioxygen. The data from those two groups, obtained over a narrower concentration range, 0.25−1 mM and 0.13−0.55 mM, respectively, show, as expected for a radical mechanism, no dependence on the peroxynitrite concentration. They determined *only* nitrite and only by the Griess method. We used ion chromatography, polarography, and the Griess method and found that there is a dependence on the peroxynitrite concentration. There is good agreement between the nitrite results from ion chromatography and polarography. Together with the nitrate results, we can account for all peroxynitrite. The nitrite results obtained with the Griess method were more variable. Hurst and co-workers[8,9] support their proposal with a simulation based on known rate constants. The rate constant used for eq 11:

$$\mathrm{ONOO^-} \rightarrow \mathrm{NO^\bullet} + \mathrm{O_2^{\bullet -}} \tag{11}$$

$k = 0.017\ \mathrm{s^{-1}}$ [21] is in doubt as we have recently shown.[22] Yet, their model predicts dioxygen yields reasonably well in the absence of nitrite and hexacyanoferrate(III). However, we note that at pH 9 (the only pH where a comparison is possible) and with nitrite present, yields of nitrite predicted by the model are higher, while with hexacyanoferrate(II) present, they are lower than the experimental values. A mechanism[8,9] that relies on two homolyses and many subsequent radical reactions to arrive at a ratio of nitrite to dioxygen of 2:1 is more complex than the generally accepted peroxyacid mechanism.[19,20] Our data support a radical-free mechanism rather than a free-radical mechanism.

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**Supporting Information Available:** A tabulation of nitrite results from peroxynitrite decay as a function of temperature and pH from which Figure 1 was constructed (Tables S1 and S2) (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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