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Formation of Peroxynitrite from the Oxidation of Hydrogen Peroxide by Nitrosonium Ion (NO^+): A Pulse Radiolysis Study

Sara Goldstein* and Gidon Czapski

Department of Physical Chemistry, The Hebrew University of Jerusalem, Jerusalem 91904, Israel

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The rate constant of the reaction of $^\bullet N_3$ with $^\bullet NO$ has been determined to be $(4.4 \pm 0.5) \times 10^9$ M^{-1} s^{-1} using the pulse radiolysis technique at pH 7.6−8.3 and 21 °C. The reaction of $^\bullet N_3$ with $^\bullet NO$ takes place through an inner-sphere electron-transfer mechanism yielding N_3NO as an intermediate, which subsequently decays to N_2O and N_2. Peroxynitrite was formed when H_2O_2 was added to the $^\bullet N_3/^\bullet NO$ system at pH 5.8−8.3. The maximum yield of peroxynitrite, which was obtained at $[H_2O_2] > 0.2$ M, was ∼34% of the initially produced $^\bullet N_3$, indicating that N_3NO does not react directly with H_2O_2. We conclude that, in the presence of high concentrations of H_2O_2, ∼64% of the N_3NO decomposes into N_2O and N_2, whereas the remaining 34% yields NO^+ or $H_2NO_2^+$, which subsequently reacts with H_2O_2 to form peroxynitrite. The comparison of our kinetic results with those obtained previously in the $H^+/HNO_2/H_2O_2$ system shows that the nitrosating species in both systems differ. As $H_2NO_2^+$ is the precursor of NO^+ in the $H^+/HNO_2/H_2O_2$ system, we conclude that the reactive intermediate in our system is most probably NO^+. From the dependence of the yield of peroxynitrite on $[H_2O_2]$, the ratio between the rate constants of the reactions of NO^+ with H_2O_2 and H_2O was determined to be 65 M^{-1}. These rate constants were estimated to be $k_7 > 3 \times 10^8$ and $k_{-4}[H_2O] > 4.6 \times 10^6$ s^{-1}, respectively.

Introduction

Nitric oxide has become in the last few years one of the most studied and fascinating molecules in biological chemistry. $^\bullet NO$ is generated from L-arginine by the enzyme NO synthase[1] and is involved in a large number of diverse biological processes.[1−4] Biologically, the important reactions of $^\bullet NO$ are those with oxygen in its various redox forms and with transition metal ions.[5]

Excess production of $^\bullet NO$ is toxic.[6−9] The toxicity of $^\bullet NO$ has been partially attributed to the formation of peroxynitrite ($ONOO^-$).[9−11] The latter is a potent oxidant that oxidizes a large variety of biomolecules such as sulfhydryls,[12] lipids,[13] enzymes,[14] and DNA.[15]

The formation of peroxynitrite can take place through the reaction of $^\bullet NO$, NO^-, or NO^+ ($H_2NO_2^+$) with $O_2^{\bullet-}$, O_2, and H_2O_2, respectively. The occurrence of the reaction of $^\bullet NO$ with superoxide has been demonstrated in many biological systems.[9,11] The reaction is diffusion-controlled with $k_1 = (4.3-6.7) \times 10^9$ M^{-1} s^{-1}.[16,17]

$$^\bullet NO + O_2^{\bullet -} \rightarrow ONOO^- \tag{1}$$

Nitroxyl anion (NO^-) can form peroxynitrite through its reaction with molecular oxygen.[18] There is no evidence that this reaction takes place in biological systems. The rate constant of reaction 2 has not yet been determined.

$$NO^- + O_2 \rightarrow ONOO^- \tag{2}$$

Nitrosonium ion (NO^+) is a well-known chemical entity. It is formed in strong acid solutions of nitrous acid via eqs 3 and 4.

$$H^+ + HNO_2 \rightleftharpoons H_2NO_2^+ \tag{3}$$

$$H_2NO_2^+ \rightleftharpoons NO^+ + H_2O \tag{4}$$

The equilibrium constant of reaction 5 has been determined

$$H^+ + HNO_2 \rightleftharpoons H_2O + NO^+ \tag{5}$$

spectrophotometrically by measuring HNO_2 and NO^+ in $HClO_4$ to be $K_5 = K_3K_4 = 3 \times 10^{-7}$ M^{-1}.[19] There was no spectroscopic evidence for the formation of $H_2NO_2^+$, and its yield could not exceed 5% under these conditions.[19] Nevertheless, kinetically, the nitrous acidium ion remains as a possible nitrosating agent. The very small value of K_5 indicates that

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* To whom all correspondence should be addressed.

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NO^+, and most probably $H_2NO_2^+$, cannot have any biological relevance under weakly acidic and physiological conditions.

Anbar and Taube[20] demonstrated that peroxynitrite is formed as an intermediate during the reaction of nitrite with H_2O_2 at pH 4−6 and 25 °C. The kinetic data were consistent with both NO^+ or "an isomeric change in $H_2NO_2^+$" as the nitrosating entities, though NO^+ seemed at that time a more attractive possibility.[20]

$$H_2NO_2^+ + H_2O_2 \rightarrow ONOOH + H^+ + H_2O \qquad (6)$$

or

$$NO^+ + H_2O_2 \rightarrow ONOOH + H^+ \qquad (7)$$

In this study, we used the pulse radiolysis technique to generate $^{\bullet}N_3$ in order to oxidize $^{\bullet}NO$ and to study the reaction of the oxidized species with H_2O_2.

Experimental Section

Chemicals. All chemicals were of analytical grade and were used as received. Solutions were prepared with deionized water that was distilled and purified using a Milli-Q water purification system. Nitric oxide, C.P., was bought from Matheson Gas Products. $^{\bullet}NO$ was purified by passing it through a series of scrubbing bottles containing 50% NaOH and purified water in this order. The solutions in the traps were first deaerated by purging them with helium for 1 h. Nitric oxide solutions were prepared in gas-tight syringes by purging first 1 mM phosphate buffer solutions with helium to remove O_2, followed by bubbling for 30 min with $^{\bullet}NO$. The $^{\bullet}NO$-saturated solutions (1.8 mM at 21 °C and 690 mmHg,[21] which is the barometric pressure in Jerusalem) were stored in syringes and subsequently diluted with N_2O-saturated solutions to the desired concentrations by the syringe technique. All experiments were carried out at 21 °C.

The concentration of H_2O_2 was determined with the super-Fricke dosimeter (10 mM Fe^{II} in 0.8 N H_2SO_4) using $\epsilon_{302}(Fe^{III}) = 2200\ M^{-1}\ cm^{-1}$.

Methods. Pulse radiolysis experiments were carried out with the Varian 7715 linear accelerator with 5 MeV electrons pulses of 0.1−1.5 μs and 200-mA current. The dose per pulse was 3−29 Gy, respectively, and was determined with either the thiocyanate dosimeter (10 mM KSCN in N_2O-saturated water) or the hexacyanoferrate(II) dosimeter (5 mM $K_4Fe(CN)_6$ in N_2O-saturated water) using $G\epsilon((SCN)_2^{\bullet-}) = 5.0 \times 10^4\ M^{-1}\ cm^{-1}$ at 475 nm and $G\epsilon(Fe(CN)_6^{3-}) = 6.7 \times 10^3\ M^{-1}\ cm^{-1}$ at 420 nm.[22] A 200-W Xe−Hg lamp produced the analyzing light. Appropriate filters were used to minimize photochemistry. Irradiation was carried out in a 4-cm spectrosil cell using three light passes.

Results and Discussion

Reaction of $^{\bullet}N_3$ with $^{\bullet}NO$. When N_2O-saturated solutions ($[N_2O] = 0.025$ M) containing azide are irradiated at pH >3, the following reactions take place:

$$H_2O \xrightarrow{\gamma} e^-_{aq}\ (2.6),\ ^{\bullet}OH\ (2.7),\ H^{\bullet}\ (0.6),\ H_2\ (0.45),\ H_2O_2\ (0.7),\ H_3O^+\ (2.6) \qquad (8)$$

The numbers in parentheses are *G* values, which represent the number of molecules formed per 100 eV energy absorbed by pure water.

$$e^-_{aq} + N_2O \rightarrow N_2 + OH^- + OH^{\bullet} \qquad k_9 = 9.1 \times 10^9\ M^{-1}\ s^{-1}\ {}^{23} \qquad (9)$$

$$^{\bullet}OH + N_3^- \rightarrow {}^{\bullet}N_3 + OH^- \qquad k_{10} = 1.2 \times 10^{10}\ M^{-1}\ s^{-1}\ {}^{23} \qquad (10)$$

$$H^{\bullet} + N_3^- \rightarrow HN_3^- \qquad k_{11} = 2.9 \times 10^9\ M^{-1}\ s^{-1}\ {}^{23} \qquad (11)$$

The azide radical is a strong one-electron oxidizing agent with a reduction potential of 1.3 V vs NHE.[24] The azide radical exhibits an optical absorption in the UV region with a sharp maximum at 274−277 nm ($\epsilon = 1400-2025\ M^{-1}\ cm^{-1}$).[25,26]

Under our experimental conditions (N_2O-saturated solutions containing 20 mM azide at pH 7.6−8.3 (2 mM phosphate buffer), a slit width 0.5 mm, and a dose of 8.6 Gy), we determined $\epsilon_{275}(^{\bullet}N_3) = (1690 \pm 40)\ M^{-1}\ cm^{-1}$. The decay of $^{\bullet}N_3$ was second order and $2k_{12} = (8.1 \pm 0.6) \times 10^9\ M^{-1}\ s^{-1}$ in agreement with previous results.[25,26]

$$^{\bullet}N_3 + {}^{\bullet}N_3 \rightarrow 3\ N_2 \qquad (12)$$

In the presence of $^{\bullet}NO$, reactions 13−15 compete with reactions 9−11.

$$e^-_{aq} + {}^{\bullet}NO \rightarrow NO^- \qquad k_{13} = 3.1 \times 10^{10}\ M^{-1}\ s^{-1}\ {}^{23} \qquad (13)$$

$$^{\bullet}OH + {}^{\bullet}NO \rightarrow HONO \qquad k_{14} = 2.0 \times 10^{10}\ M^{-1}\ s^{-1}\ {}^{23} \qquad (14)$$

$$^{\bullet}H + {}^{\bullet}NO \rightarrow HNO \qquad k_{15} = 1.1 \times 10^{10}\ M^{-1}\ s^{-1}\ {}^{23} \qquad (15)$$

If NO^- is formed, it will react rapidly with $^{\bullet}NO$ to form $N_2O_2^-$ (and subsequently $N_3O_3^-$), which can easily be detected as $\epsilon_{380}(N_2O_2^-) \sim 3000\ M^{-1}\ cm^{-1}$ and $\epsilon_{380}(N_3O_3^-) \sim 4000\ M^{-1}\ cm^{-1}$.[27,28] Under the conditions where $[N_3^-]/[^{\bullet}NO]$ and $[N_2O]/[^{\bullet}NO]$ are higher than 50, e^-_{aq} and $^{\bullet}OH$ were converted into $^{\bullet}N_3$, and the decay of $^{\bullet}N_3$ was first order.

$$^{\bullet}N_3 + {}^{\bullet}NO \rightarrow \text{products} \qquad (16)$$

The observed first-order rate constant was linearly dependent on $[^{\bullet}NO]_o$ (Figure 1), and k_{16} was determined from the slope of the line in Figure 1 to be $(4.4 \pm 0.5) \times 10^9\ M^{-1}\ s^{-1}$.

Solutions containing 0.02 M N_2O, 3.6×10^{-4} M $^{\bullet}NO$, and 0.016−0.024 M azide at pH 7.6 (1.6 mM phosphate buffer) were pulse-irradiated with a total dose of 350−475 Gy. The light absorption of the solution was monitored before and after the irradiation. We found that the yield of nitrite was very low, 6−9%. This measurement is inaccurate due to the very small extinction coefficient of nitrite ($\epsilon_{354} = 21\ M^{-1}\ cm^{-1}$).

The reduction potential of $^{\bullet}NO/NO^+$ is 1.21 V,[24] whereas that of $^{\bullet}N_3/N_3^-$ is 1.3 V.[24] Therefore, from the thermodynamic point of view, reaction 16 can take place through an outer-sphere electron-transfer mechanism, yielding nitrite as a final product.

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$$^\bullet N_3 + {}^\bullet NO \rightarrow NO^+ + N_3^- \tag{17}$$

$$NO^+ + H_2O \rightleftharpoons H_2NO_2^+ \tag{-4}$$

$$H_2NO_2^+ \rightleftharpoons H^+ + HNO_2 \tag{-3}$$

As the yield of nitrite did not exceed 9%, the outer-sphere electron-transfer mechanism is rejected, and an inner-sphere electron-transfer mechanism should be considered.

$$^\bullet N_3 + {}^\bullet NO \rightarrow N_3NO \tag{18}$$

The formation of N_3NO as an intermediate has already been shown in the reaction between HN_3 and HNO_2 by ^{15}N tracer experiments.[29] This species can also be isolated at low temperatures.[30]

The very low yield of nitrite under our experimental conditions can be attributed to reactions 13−15. Under our experimental conditions, 5.8% of the hydrated electrons are converted into NO^- (reaction 13), 2.4−3.6% of the hydroxyl radicals are converted into nitrite (reaction 14), and 6.7−9.7% of the hydrogen atoms are converted into NO^- (reaction 15). NO^- reacts rapidly with $^\bullet NO$ to yield $N_2O_2^-$ (and subsequently $N_3O_3^-$). The final products of this process are N_2O and NO_2^-.[27,28] Thus, the total yield of nitrite due to reactions 13−15 is 4.8−5.5%, which is within experimental error identical to the measured yields of 6−9%. This indicates that the decomposition of N_3NO takes place mainly via reaction 19 and that there is no appreciable hydrolysis N_3NO in aqueous solutions in agreement with previous results.[31]

$$N_3NO \rightarrow N_2O + N_2 \tag{19}$$

$^\bullet N_3$/$^\bullet NO$/H_2O_2 System. When H_2O_2 ($pK_a = 11.8$) is added to N_2O-saturated solutions containing azide and $^\bullet NO$, reactions 20−22 may compete with reactions 9, 10, and 16. (The rate

$$e^-_{aq} + H_2O_2 \rightarrow {}^\bullet OH + OH^- \qquad k_{20} = 1.2 \times 10^{10}\ M^{-1}\ s^{-1\ 23} \tag{20}$$

$$^\bullet OH + H_2O_2 \rightarrow H^+ + O_2^{\bullet -} + H_2O \qquad k_{21} = 3.5 \times 10^{7}\ M^{-1}\ s^{-1\ 23} \tag{21}$$

$$^\bullet N_3 + HO_2^- \rightarrow N_3^- + H^+ + O_2^{\bullet -} \qquad k_{22} = 3.2 \times 10^{9}\ M^{-1}\ s^{-1\ 23} \tag{22}$$

constant of $^\bullet N_3$ with H_2O_2 is lower than $5 \times 10^6\ M^{-1}\ s^{-1}$).[23]

When $[N_3^-]/[^\bullet NO]$ and $[N_2O]/[^\bullet NO]$ are higher than 50, $[^\bullet NO] = 60{-}360\ \mu M$, $[H_2O_2] < 0.2$ M, and pH <8.5, most of the hydroxyl radicals and the hydrated electrons are converted into $^\bullet N_3$, which subsequently reacts with $^\bullet NO$ and not with H_2O_2. Under these conditions, the following features were observed:

(i) A fast formation of an absorption with maximum at 300 ± 3 nm (Figure 2). The formation rate of the absorbance was independent of $[N_3^-]$ and $[H_2O_2]$ but dependent on $[^\bullet NO]_o$, indicating that the rate-determining step is the reaction of $^\bullet N_3$ with $^\bullet NO$

(ii) The absorption yield increased with $[H_2O_2]$, reaching a plateau value at $[H_2O_2] > 0.2$ M (Figure 3).

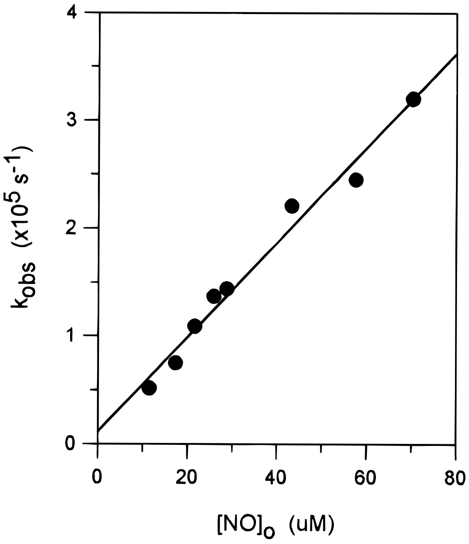

Figure 1. Observed first-order rate constant of the decay of $^\bullet N_3$ as a function of $[^\bullet NO]_o$ in 0.02 M N_2O and 0.02 M azide at pH 8.3 (1.6 mM phosphate buffer). The dose was 3.2−5.0 Gy.

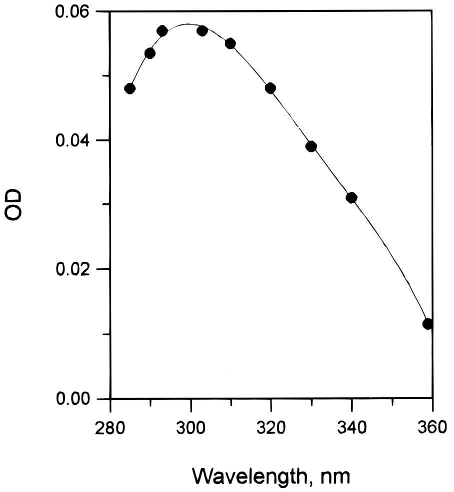

Figure 2. Absorption spectrum obtained 100 μs after the end of the pulse. The solution contained 0.02 M N_2O, 0.019 M azide, 144 μM $^\bullet NO$, and 0.1 M H_2O_2 at pH 8.1 (1.6 mM phosphate buffer). The optical path length was 12.3 cm, and the dose was 14.6 Gy.

(iii) The absorption yield was independent of $[N_3^-]$ ($[N_3^-] = 5 \times 10^{-3}{-}0.2$ M) as observed in the presence of 0.05 M H_2O_2 at pH 8.1 (1.6 mM phosphate buffer).

(iv) The absorption yield was pH-dependent, resulting in an apparent $pK_a = 7$ (Figure 4).

(v) The decay of the absorbance followed first-order kinetics. The observed first-order rate constant decreased with the increase in pH: 0.95, 0.63, 0.32, and 0.11 s^{-1} at pH 5.8, 6.4, 7, and 7.3, respectively, at 21 °C. At pH >7.5, the decay was subject to photolysis and could not be measured.[17,32]

These results demonstrate that the species formed in the $^\bullet N_3$/$^\bullet NO$/H_2O_2 system is peroxynitrite. This conclusion was based on the known properties of peroxynitrite: (a) peroxynitrous acid has a $pK_a = 6.8$ [10]; (b) the maximum absorption of $ONOO^-$ is at 302 nm ($\epsilon = 1670 \pm 50\ M^{-1}\ cm^{-1}$),[33] whereas ϵ_{302}(ONOOH) ~200 $M^{-1}\ cm^{-1}$;[17,32] (c) $ONOO^-$ is stable whereas ONOOH decays fast to nitrate with $k_d \sim 1.3\ s^{-1}$ at 25 °C.[10]

The maximum yield of peroxynitrite was calculated from Figure 3 to be ~30% of the initially produced $^\bullet N_3$ using $\epsilon_{303} =$

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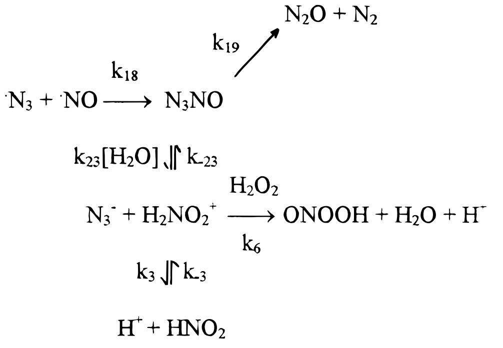
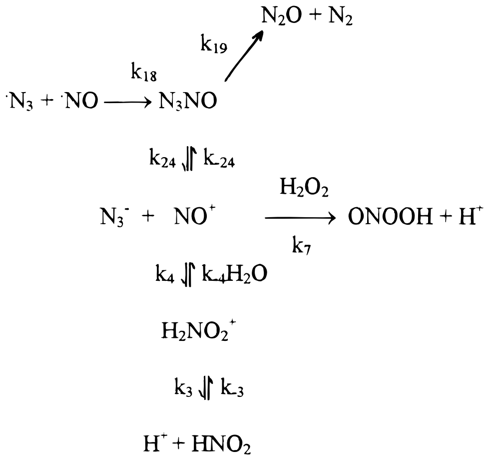
1670 M^{-1} cm^{-1} and $pK_a = 6.8$. This result supports our previous conclusion that reaction 16 proceeds through an inner-sphere electron-transfer mechanism. If NO^+ would have been formed via reaction 17, one would expect a 100% yield of peroxynitrite at infinite H_2O_2, where reaction 7 competes efficiently with the hydrolysis of NO^+. In addition, N_3NO cannot be the oxidizing species, as in this case a 100% yield of peroxynitrite would also be expected at infinite H_2O_2.

Our experimental observations are in accord with the formation of peroxynitrite through the reaction of H_2O_2 with $H_2NO_2^+$ (mechanism I) or through the reaction of H_2O_2 with NO^+ (mechanisms II).

mechanism I



mechanism II



The yield of peroxynitrite was independent of $[N_3^-] = 5 \times 10^{-3}$–0.2 M in the presence of 50 mM H_2O_2. Therefore, in mechanism I, reaction −23 can be neglected, and the competition for $H_2NO_2^+$ is between reactions −3 and 6, where $OD_{303}(max)$ is the maximum yield of peroxynitrite at infinite H_2O_2:

$$OD_{303} = OD_{303}(max)k_6[H_2O_2]/(k_6[H_2O_2] + k_{-3}) \quad (25)$$

If mechanism II applies, reaction −24 can be neglected, and the competition for NO^+ is between reactions −4 and 7.

$$OD_{303} = OD_{303}(max)k_7[H_2O_2]/(k_7[H_2O_2] + k_{-4}[H_2O]) \quad (26)$$

A plot of $1/OD_{303}$ as a function of $1/[H_2O_2]$ yields a straight line (Figure 5), where 1/intercept = $OD_{303}(max) = 0.064$ and intercept/slope = I/S = 65 M^{-1}, which equals k_6/k_{-3} (mechanism I) or $k_7/k_{-4}[H_2O]$ (mechanism II). As $OD_{303}(max) = 0.064$ at infinite H_2O_2 and at pH 8 ($[{}^\bullet N_3]_o = 9.75$ μM, l = 12.3 cm), the maximum yield of peroxynitrite is calculated to be 34% of the initially produced ${}^\bullet N_3$, using $pK_a = 6.8$ and $\epsilon_{303} = 1670$ M^{-1} cm^{-1}. This value is somewhat higher than that of the 30% calculated from the plateau region of Figure 3 and shows that k_{19}/k_{23} or $k_{19}/k_{24} = 2$.

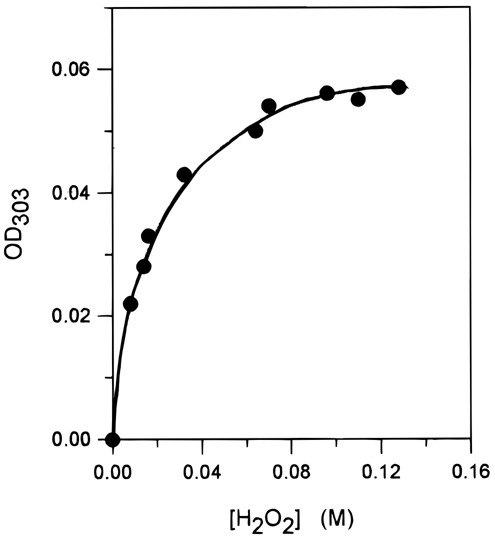


Figure 3. Absorbance at 303 nm measured 100 μs after the end of the pulse as a function of $[H_2O_2]$. Solutions contained 0.023 M N_2O, 0.019 M azide, and 72–360 μM ${}^\bullet NO$ at pH 8.0 (1.8 mM phosphate buffer). The optical path length was 12.3 cm, and the dose was 14.6 Gy.

The rate-determining step of the nitrosation process when $[{}^\bullet NO] = 60 - 140$ μM and $[H_2O_2] \geq 8$ mM is reaction 18 ($k_{19}[N_3NO]$ and $k_6[H_2NO_2^+][H_2O_2]$ or $k_7[NO^+][H_2O_2]$ exceed $k_{18}[{}^\bullet NO][{}^\bullet N_3]$), and hence, $k_{19} > 1 \times 10^6$ s^{-1} and k_6 or $k_7 > 3 \times 10^8$ M^{-1} s^{-1}.

Anbar and Taube[20] measured the rate of the disappearance of nitrite as a function of H_2O_2 at pH 4–6 (0.3 M phosphate buffer) and 25 °C. Benton and Moore[34] measured the formation rate of ONOOH as a function of H_2O_2 in the presence of nitrous acid at pH <2 and 0 °C. In both studies the rate law was found to obey eq 27, where *a*, *b*, and *c* are constants.

$$\text{rate} = \frac{a[H^+][HNO_2]\,[H_2O_2]}{b + c[H_2O_2]} = k_{obs}[HNO_2] \quad (27)$$

A plot of $[H^+]/k_{obs}$ as a function of $1/[H_2O_2]$ yielded a straight line with intercept/slope = I/S = c/b = 2.32 [34] to 2.4 M^{-1} [20] and 1/I = a/c = 617 [34] to 2.9×10^3 M^{-1} s^{-1}.[20] The rate law is consistent with either NO^+ or $H_2NO_2^+$ as the reactive intermediates. If NO^+ is the nitrosating species (reactions 3, 4, and 7), rate equation 27 is obtained where $a = k_7k_4K_3$, $b = k_{-4}[H_2O]$ and $c = k_7$. If $H_2NO_2^+$ is the reactive intermediate (reactions 3 and 6), rate eq 27 is obtained where $a = k_6k_3$, $b = k_{-3}$ and $c = k_6$. The kinetic results in the $H^+/HNO_2/H_2O_2$ cannot distinguish between NO^+ and $H_2NO_2^+$ (or "an isomeric change in $H_2NO_2^+$"[20]) as noted by Anbar and Taube.[20] Benton and Moore[34] concluded wrongly that NO^+ is the reactive intermediate, although they noted that at low concentrations of H_2O_2 it is impossible to distinguish between NO^+ and $H_2NO_2^+$.

The kinetic results obtained in the $H^+/HNO_2/H_2O_2$ and ${}^\bullet N_3/{}^\bullet NO/H_2O_2$ systems can fit the mechanisms where either $H_2NO_2^+$ (or an isomeric change in $H_2NO_2^+$ [20]) or NO^+ is the nitrosating species. In our study, the competition ratio equals 65 M^{-1}, whereas in the $H^+/HNO_2/H_2O_2$ system it equals 2.32 [34] to 2.4 M^{-1}.[20] This shows that the reactive intermediates in these two systems differ.

Due to this difference, we argue that $H_2NO_2^+$ (or its isomeric form) cannot be the reactive species in our system. If this would

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(34) Benton, D. J.; Moore, P. *J. Chem. Soc. A* **1970**, 3179.

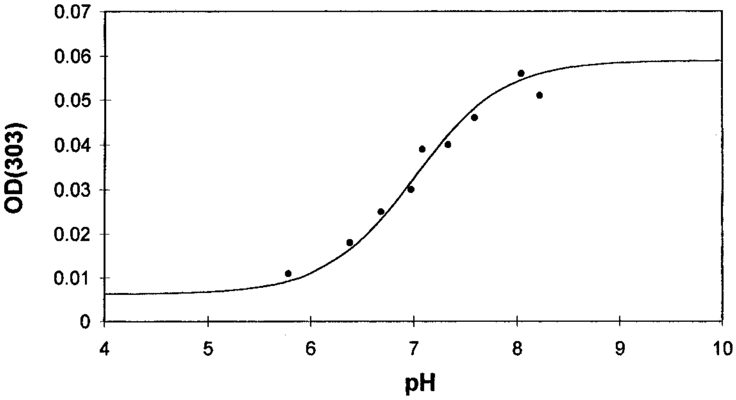


Figure 4. Absorbance at 303 nm as measured 100 μs after the end of the pulse as a function of pH. Solutions contained 0.023 M N_2O, 0.019 M azide, 144 μM $^{\bullet}NO$, and 0.1 M H_2O_2. The optical path length was 12.3 cm, and the dose was 14.6 Gy. The solid line is the fit of the data to $\epsilon_{303}(ONOO^-) = 1670$ M^{-1} cm^{-1}, $\epsilon_{303}(ONOOH) = 178$ M^{-1} cm^{-1}, and $pK_a = 7.0$.

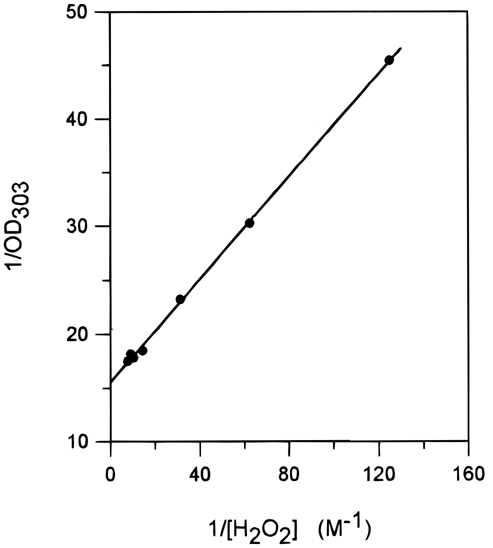


Figure 5. Double-reciprocal plot of the change in the absorbance at 303 nm with varying the concentrations of H_2O_2 in the presence of 0.023 M N_2O, 0.019 M azide, and 72−360 μM $^{\bullet}NO$ at pH 8.0 (1.8 mM phosphate buffer). The optical path length was 12.3 cm, and the dose was 14.6 Gy.

be the case, the same competition ratio would be expected in both systems as $H_2NO_2^+$ is the precursor of NO^+ in the $H^+/HNO_2/H_2O_2$ system, and we have determined above that k_6 exceeds 3×10^8 M^{-1} s^{-1}. NO^+ will be formed in the $H^+/HNO_2/H_2O_2$ system provided that $k_4 > k_6[H_2O_2]$. In such a case, $H_2NO_2^+$ cannot be the nitrosating entity in our system, but NO^+, and the same competition ratio is expected in both systems. This conclusion is not affected if one assumes that the nitrosating intermediate in the $H^+/HNO_2/H_2O_2$ system is the isomeric form of $H_2NO_2^+$ as suggested by Anbar and Taube[20] (see footnote 35).

We conclude that the nitrosating species in our system is most probably NO^+, and therefore $k_7/k_{-4}[H_2O] = 65$ M^{-1}, $k_7 > 3 \times 10^8$ M^{-1} s^{-1}, and $k_{-4}[H_2O] > 4.6 \times 10^6$ s^{-1}. The value of k_7 cannot exceed the diffusion-controlled limit, and therefore $3 \times 10^8 < k_7 < 1 \times 10^{10}$ M^{-1} s^{-1}, and $4.6 \times 10^6 < k_{-4}[H_2O < 1.5 \times 10^8$ s^{-1}. As 0.2 M azide had no effect on the yield of peroxynitrite in the presence of 50 mM H_2O_2, we calculate that $8 \times 10^7 < k_{-24} < 3 \times 10^9$ M^{-1} s^{-1}.

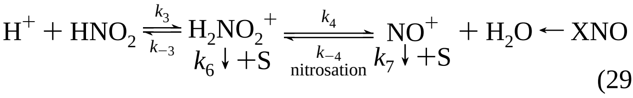
The nitrosating entity in the $H^+/HNO_2/H_2O_2$ system is most probably $H_2NO_2^+$ and therefore, $I/S = k_6/k_{-3} = 2.32{-}2.4$ M^{-1} and $1/I = k_3 = 617{-}2900$ M^{-1} s^{-1}.[20,34,36] These results demonstrate that $H_2NO_2^+$ is a strong acid, as rates of protonation of strong acids are considerably lower than the diffusion-controlled limit.

Bunton and Stedman[31] studied the reaction of HN_3 with HNO_2 in [^{18}O]water, and showed that the reaction takes place via the oxidation of azide ion by $H_2NO_2^+$.

$$H^+ + HNO_2 \rightleftharpoons H_2NO_2^+ \overset{N_3^-}{\rightleftharpoons} N_3NO + H_2O \rightarrow N_2O + N_2 \tag{28}$$

Their conclusion does not contradict ours, as in their system $H_2NO_2^+$ is scavenged by N_3^- before NO^+ is formed. In our system, N_3NO is initially produced and dissociates into NO^+ and N_3^-. Therefore, if high concentrations of a potential substrate are present, e.g., H_2O_2, it will scavenge NO^+ and $H_2NO_2^+$ will not be formed.

We conclude that both NO^+ and $H_2NO_2^+$ can oxidize rapidly H_2O_2 and N_3^- as well as other substrates.[31,37] The nature of the reactive intermediate will depend on the particular system.



$$H^+ + HNO_2 \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \underset{k_6 \downarrow +S}{H_2NO_2^+} \underset{\substack{k_{-4}\\ \text{nitrosation}}}{\overset{k_4}{\rightleftharpoons}} \underset{k_7 \downarrow +S}{NO^+} + H_2O \leftarrow XNO \tag{29}$$

If the nitrosation starts from the left through the reaction of H^+ with HNO_2, $H_2NO_2^+$ will most probably be the reactive intermediate, provided that $k_6[S]$ exceeds k_4. In cases where NO^+ is initially formed, e.g., from N_3NO or other NO^+-releasing

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(35) Anbar and Taube[20] noted that the rate law for the exchange of oxygen between nitrite and water can fit both NO^+ or an isomeric change in $H_2NO_2^+$. Therefore, the nitrosating intermediate in their system in the presence of H_2O_2 can be $H_2NO_2^+$, its isomeric form, or NO^+. The rate law for the decay of H_2O_2 given in ref 20 has the form of our eq 27 and can fit these three possibilities. We conclude that in our system NO^+ is the nitrosating intermediate whereas in the $H^+/HNO_2/H_2O_2$ system it is $H_2NO_2^+$ or its isomeric form. $H_2NO_2^+$ cannot be the reactive intermediate in our system and the isomeric form in the $H^+/HNO_2/H_2O_2$ system because $H_2NO_2^+$ is the precursor of its isomeric form.

(36) Halfpenny and Robinson (*J. Chem. Soc. A* **1952**, 928.) studied the reaction of HNO_2 with H_2O_2 at 19 °C and obtained that $-d[H_2O_2]/dt = k_{obs}[H^+][HNO_2][H_2O_2]$ and $k_{obs} = 140$ M^{-2} s^{-1}. According to the mechanism given by reactions 3 and 6, rate eq 27 reduces to the observed rate when $k_{-3} > k_6[H_2O_2]$, and hence, $k_{obs} = k_6K_3 = 140$ M^{-2} s^{-1}. As $k_6/k_{-3} = 2.4$ M^{-1},[20,34] $k_3 = 60$ M^{-1} s^{-1}, which is more than 1 order of magnitude lower than the values determined in refs 20 and 34. The source for this discrepancy may be the following: Halfpenny and Robinson studied the reaction of HNO_2 with H_2O_2 in the presence of relatively high concentrations of H_2O_2 (≤ 0.176 M), where k_{-3} does not exceed $k_6[H_2O_2]$. Therefore, $k_6[H_2O_2]$ cannot be neglected, and 140 M^{-2} s^{-1} equals $k_6K_3/(k_{-3} + k_6[H_2O_2])$, which is lower than k_6K_3.

(37) Ridd, J. H. *Adv. Phys. Org. Chem.* **1978**, *16*, 1.

compounds,[5,37,38] it will most probably react with the substrate, and $H_2NO_2^+$ will not be formed, unless $k_{-4}[H_2O]$ exceeds $k_7[S]$.

Conclusions

The azide radical reacts with $^{\bullet}NO$ to yield N_3NO, which decomposes mainly into N_2O and N_2. In the presence of H_2O_2, peroxynitrite is formed. The maximum yield of peroxynitrite at $[H_2O_2] > 0.2$ M is 34%, indicating that N_3NO does not react directly with H_2O_2. The kinetic results suggest that N_3NO may either hydrolyze to $H_2NO_2^+$ or dissociate into NO^+ in the presence of H_2O_2. Both intermediates are capable of nitrosating H_2O_2.

The comparison of our competition ratio with that obtained in the $H^+/HNO_2/H_2O_2$ system,[20,34] indicates that the nitrosating intermediates in these systems differ. As $H_2NO_2^+$ is the precursor of NO^+ in the case of $H^+/HNO_2/H_2O_2$, we conclude that the reactive species in our system is most probably NO^+, whereas in the $H^+/HNO_2/H_2O_2$ system it is $H_2NO_2^+$.

Nitrosation of a substrate by NO^+ or $H_2NO_2^+$ will take place only if the rate of these reactions competes efficiently with the hydrolysis of NO^+ or with the dissociation of $H_2NO_2^+$. Even if the rate constant of the nitrosation is diffusion-controlled, the concentrations of the substrate in the case of NO^+ should exceed 0.5 mM as the half-life of NO^+ is shorter than 0.15 μs. Such high concentrations are not always present in biological systems. Therefore, we suggest that nitrosation *in vivo* may take place via NOX, where X^- represents an ion that reacts fast with $H_2NO_2^+$ or NO^+[37,38] and that is present at relatively high concentrations, e.g., chloride. This mechanism will apply only in cases where the rates of the decomposition and/or hydrolysis of NOX are considerably slower than those of the dissociation of $H_2NO_2^+$ and the hydrolysis of NO^+.

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(38) Stedman, G. *Adv. Inorg. Chem. Radiochem.* **1979**, *22*, 113.