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Chemistry of Peroxynitrites as Compared to Peroxynitrates

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1. Introduction

Peroxynitrous acid (ONOOH) and peroxynitric acid (O_2NOOH) are two small inorganic nitrogen-containing hydroperoxides, which have been thoroughly investigated in the gas phase[1−11] as well as in aqueous solution.[12−21] While suggested by several workers as a likely and even expected intermediate,[3,5,7,8] until recently, ONOOH was not observed in the gas phase.

As is well-known, the reaction of $HO_2^{\bullet}$ with $^{\bullet}NO$ is a major producer of the $^{\bullet}OH$ radical, which is considered as the oxidative "work-horse" in the atmosphere (reaction 1).[22]

$$HO_2^{\bullet}{}_{(g)} + {}^{\bullet}NO_{(g)} \rightarrow {}^{\bullet}OH_{(g)} + {}^{\bullet}NO_{2(g)} \qquad (1)$$

This reaction yields the products without any observable intermediate.[2,4] On the other hand, the reaction of $^{\bullet}OH$ with $^{\bullet}NO_2$ yields nitric acid (reaction 2), which is an important reservoir of $^{\bullet}OH$ radicals.[4,5]

$${}^{\bullet}OH_{(g)} + {}^{\bullet}NO_{2(g)} + M \rightarrow HNO_{3(g)} + M \qquad (2)$$

The pressure dependence of reaction 2 has indicated, for a long time, a second channel, namely reaction 3, whose contribution appeared to increase with [M], the concentration of the inert bath gas.[5]

$${}^{\bullet}NO_{2(g)} + {}^{\bullet}OH_{(g)} + M \rightleftharpoons ONOOH_{(g)} + M \qquad (3)$$

Recently, ONOOH was detected at high temperatures and pressures and from the data, the enthalpy of reaction 3 at 0 K was determined to be $\Delta H°(3) = -19.8 \pm 0.3$ kcal/mol.[6] This species is not seen in the gas phase at ambient pressure and temperature, presumably because its lifetime is too short, probably a few milliseconds.

In contrast to the elusive nature of gaseous ONOOH, its formation in aqueous solution was unambiguously proven via both radical coupling reactions 4[15] and 5.[17]

$${}^{\bullet}NO_2 + {}^{\bullet}OH \rightarrow ONOOH \qquad (4)$$

$${}^{\bullet}NO + HO_2^{\bullet} \rightarrow ONOOH \qquad (5)$$

ONOOH is a rather strong acid, and its pK_a for deprotonation to form peroxynitrite ($ONOO^-$) was found to be 6.5−6.8.[15,17,18,23] Hence, at physiological pH the dominant form should be peroxynitrite,

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Sara Goldstein received her Ph.D. in Chemistry from the Hebrew University of Jerusalem, Israel. Her early interests were in radiation chemistry and properties of free radicals, particularly $O_2^{\bullet -}$. Currently, her interests are in the chemistry of reactive nitrogen and oxygen species of possible biological relevance, as well as in the chemistry of cyclic nitroxides, which effectively protect biological systems against free radical-induced damage. At present, Prof. Goldstein is a Senior Research Associate in the Department of Physical Chemistry at the Hebrew University.

Dr. Johan Lind is currently Assistant Professor in Nuclear Chemistry at the Royal Institute of Technology in Stockholm, Sweden. His fields of specialization are chemical kinetics, electron transfer, and unstable intermediates. Presently, his work is focused on small molecules carrying oxygen–nitrogen bonds.

$ONOO^-$. In biological systems, reaction 6, that is, the coupling between $^{\bullet}NO$ and $O_2^{\bullet -}$, was suggested to be the most probable source of peroxynitrite,[24] and ever since, this compound has been implicated as an important toxin in various diseases.[25-28]

$$^{\bullet}NO + O_2^{\bullet -} \rightarrow ONOO^- \qquad (6)$$

While the formation of peroxynitrite in water was ascertained, its homolysis to yield $^{\bullet}OH$ and $^{\bullet}NO_2$ radicals (reaction −4),[24,29,30] previously demonstrated by means of product and selectivity analysis, has become a matter of controversy during the past decade. This situation arose mainly as a result of an influential thermochemical estimation,[31] which predicted a vanishingly small yield for free radicals. In the meantime, results from studies with spin methods such as EPR[32] and CIDNP[33] strongly suggested the homolysis of ONOOH into radicals. However, given that none of these methods was able to quantify the yield of the radicals, the possibility of the main path of ONOOH decomposition being a nonradical isomerization could not be excluded. The above thermochemical estimate was subsequently rebutted,[34] and the rebuttal duly rerebutted[35] without a firm conclusion having been arrived at. This controversy pointed to the need for an accurate *experimental* determination of the thermochemical parameters of ONOOH and $ONOO^-$. In the ensuing years, several critical experiments, both thermokinetic and by means of product analysis, were instrumental in establishing the currently accepted mechanism of ONOOH decay. Accordingly, ONOOH homolyzes along the O−O bond to yield a geminate pair of $^{\bullet}OH$ and $^{\bullet}NO_2$ in equilibrium with the reactant. Approximately 30% of the geminate pair diffuses out of the solvent cage to form free $^{\bullet}OH$ and $^{\bullet}NO_2$ radicals,[36-43] while the remainder collapses to nitric acid. Hence, the principal determinant of peroxynitrite chemistry is its exceptionally weak O−O bond. The weakness of this bond is believed to result from strong stabilization of the $^{\bullet}NO_2$ radical as compared to ordinary oxyl radicals. Another feature worth noting is that the lifetimes of all known adducts of peroxynitrite are significantly shorter than that of ONOOH.[44-47] This reflects the fact that the oxyl radical that forms as the partner to $^{\bullet}NO_2$ after homolysis of the O−O bond is usually more stabilized than the hydroxyl radical.

Dr. Gábor Merényi is a physical chemist, whose early interest centered on photochemical, particularly chemiluminescent, reaction mechanisms. Later his interest turned to the thermochemistry of unstable intermediates with a view to confirming or discarding their possible involvement in a particular reaction sequence. His most recent research concerns the thermokinetic investigation of small nitrogen and oxygen-containing species of possible biological relevance. At present, Dr. Merényi is Assistant Professor in Chemistry at the Royal Institute of Technology in Stockholm, Sweden.

Recent years have seen considerable effort in the study of the preparation, decomposition, and photochemistry of O_2NOOH, which is formed in the gas phase by the recombination of $HO_2^{\bullet}$ and $^{\bullet}NO_2$ radicals.[1,9-11]

$$HO_2{}^{\bullet}{}_{(g)} + {}^{\bullet}NO_{2(g)} \rightleftharpoons O_2NOOH_{(g)} \qquad (7)$$

O_2NOOH in aqueous solution is an even stronger acid than ONOOH, with a $pK_a = 5.9$.[16,19,20] In contrast to ONOOH, O_2NOOH homolyzes along the N−O bond to yield $HO_2^{\bullet}$ and the highly stabilized $^{\bullet}NO_2$ radical.[19,20] Peroxynitrate formation and decomposition is important in all chemical systems where $HO_2^{\bullet}/O_2^{\bullet -}$

Table 1. Activation Parameters for Peroxynitrite at 25 °C

reaction	E_a (kcal/mol)	A (s^{-1})	ΔV^* (cm^3/mol)
$ONOOH \rightarrow NO_3^- + H^+$ (pH 4)	21.6 (refs 37, 38)	1×10^{16} (refs 37, 38)	10−11 (refs 58, 59)
	22 (ref 56)	1.8×10^{15} (ref 57)	6−14 (ref 60)
	20.7 (ref 57)		6.9 (ref 61)
$ONOO^- \rightarrow 0.8NO_3^- + 0.2NO_2^- + 0.1O_2$ (pH 14)	21.7 (ref 38)	8×10^{10} (ref 38)	nd[a]
	24.1 (ref 62)	4.9×10^{12} (ref 62)	

[a] nd = not determined.

and $^{\bullet}NO_2$ are simultaneously present. This happens in atmospheric chemistry as well as in biological systems. In the former case, peroxynitric acid is formed due to photochemical reactions and is then rapidly scavenged into the aqueous phase of the clouds.[48] In biology, peroxynitrate can be formed in the lungs via the rapid reaction of $O_2^{\bullet-}$ with $^{\bullet}NO_2$, which is one of the most important toxic components of photochemical smog.[49] In addition, O_2NOO^- can form in biological systems during the decomposition of peroxynitrite.[40,50]

In the present review, we shall treat the various reaction patterns of $ONOOH/OONO^-$ and O_2NOOH/O_2NOO^- in more detail and will demonstrate the main differences between these peroxides. In this context, we shall also scrutinize the behavior of derivatives of these compounds such as alkylated peroxynitrites and peroxynitrates as well as some biologically important enzyme-based metal peroxynitrites and peroxynitrates.

2. Peroxynitrite

2.1. Reaction of $^{\bullet}NO$ with $O_2^{\bullet-}$

Blough and Zafiriou[12] demonstrated in 1985 that $^{\bullet}NO$ reacts with $O_2^{\bullet-}$ to form $ONOO^-$ in strongly alkaline solutions. Since then, several determinations of the rate constant of this reaction have been carried out, but there are disagreements in the results. The first reported rate constant for this coupling reaction was relatively low, that is, $k_6 = 3.7 \times 10^7\ M^{-1}\ s^{-1}$.[51] Later, this value was revised, and using the methods of pulse radiolysis or laser photolysis, the rate constant has been determined to be between 3.8×10^9 and $6.7 \times 10^9\ M^{-1}\ s^{-1}$.[17,43,52,53] In pulse radiolysis[17,43,53] and in laser photolysis,[52] $^{\bullet}NO$ and $O_2^{\bullet-}$ were produced in a cascade of reactions, where the rate of the slowest reaction exceeded $10^6\ s^{-1}$. Consequently, more than 90% of these two radicals were produced within less than 2 μs. Given that, at the doses employed, the lifetime of $^{\bullet}NO$ with $O_2^{\bullet-}$ during their recombination to yield $ONOO^-$ is longer than 10 μs, the recombination process is well separated from radical generation and its rate constant can be determined without distortion. When laser photolysis was used to bleach peroxynitrite and subsequently to observe the rate of restoration of its absorbance, an exceptionally high value, that is, $1.6 \times 10^{10}\ M^{-1}\ s^{-1}$, was deduced,[23,54] although with pulse radiolysis the same authors measured ca. $5 \times 10^9\ M^{-1}\ s^{-1}$;[54] that is, they essentially reproduced the values obtained in the other laboratories. We have no explanation for the significant discrepancy between the values measured by two different methods in the laboratory of Koppenol et al.[23,54] We note, however, that, contrary to the consensus around the lower value of ca. $5 \times 10^9\ M^{-1}\ s^{-1}$ in most laboratories, the high value derived from the bleaching/restoration experiments has not yet been confirmed independently. Awaiting a possible future resolution of this discrepancy, we provisionally adopt the value $k_6 = (5 \pm 1) \times 10^9\ M^{-1}\ s^{-1}$.

2.2. Mechanism and Thermochemistry of Peroxynitrite Decomposition in Aqueous Solutions

In this section, we shall review the *experimental* evidence in support of the homolysis of ONOOH into ca. 30% $^{\bullet}OH$ and $^{\bullet}NO_2$, as well as the derived reaction schemes for the decomposition of peroxynitrite under different experimental conditions.

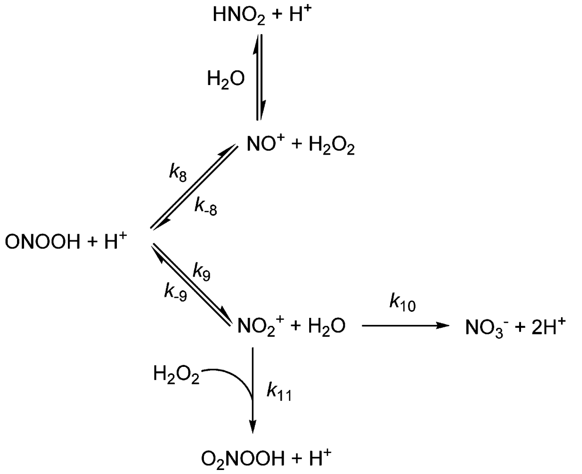
2.2.1. Activation Parameters

Peroxynitrite ion is a relatively stable species having a maximum absorption at 302 nm ($\epsilon = 1670 \pm 50\ M^{-1}\ cm^{-1}$).[13] Peroxynitrous acid decomposes relatively fast ($k_0 = 1.25 \pm 0.05\ s^{-1}$ at 25 °C)[18,23,38] and has a maximum absorption at 240 nm ($\epsilon = 770\ M^{-1}\ cm^{-1}$) with no appreciable absorption above 300 nm.[15,17] The temperature dependence of the decay of ONOOH was studied, and the activation parameters were determined to be $E_a = 20.7-22$ kcal/mol and $A = 1.8 \times 10^{15}$ to $1 \times 10^{16}\ s^{-1}$.[37,38,55−57] The value of the frequency factor is of the same order of magnitude as those found for homolysis reactions of peroxides in the gas phase and in nonpolar organic solvents, and therefore, it provides strong support for the homolysis of ONOOH. The decomposition of ONOOH was also characterized by a considerably positive activation volume of 6.9−14 cm^3/mol,[58−61] which is in favor of a bond breakage process. The temperature dependence of the decay of $ONOO^-$ at pH 14 was also studied.[38,62] The activation parameters for peroxynitrite are summarized in Table 1.

2.2.2. Gibbs' Energy of ONOOH

Recently,[41] the Gibbs' energy of ONOOH was determined to be $\Delta_f G°(ONOOH) = 7.1 \pm 0.2$ kcal/mol without involving parameters for free radicals by studying in detail the H^+-catalyzed decomposition of ONOOH. In very acidic solutions, $[H^+] = 0.1-0.75$ M, and at constant ionic strength, the decay rate of ONOOH increased linearly with increasing $[H^+]$ but *decreased* with increasing $[H_2O_2]$ at a constant $[H^+]$, approaching a limiting lower value below 0.1 M H_2O_2.[41] These observations can be interpreted by assuming the reaction of ONOOH with H^+ to form both NO^+ and NO_2^+ as intermediates (Scheme 1).

Scheme 1. Mechanism of H^+-Catalyzed Decomposition of ONOOH (Reactions 8−11)



The decay of ONOOH with and without the addition of 0.05 M H_2O_2 was followed as a function of $[H^+]$, and the rate constants for reactions 8 and 9 were determined to be 6.3 ± 0.7 and 4.3 ± 0.1 M^{-1} s^{-1}, respectively.[41] Because $k_{-9} \ll k_{10}$, regeneration of ONOOH from NO_2^+ can be completely neglected. The rate constant for formation of ONOOH was also determined when HNO_2 reacted with H_2O_2 in acidic solutions.[29,41,55,63] The formation of ONOOH is accompanied by the formation of O_2NOOH through reaction 11. However, the yield of O_2NOOH is negligible when $[H_2O_2] < 0.1$ M because $k_{10}/k_{11} = 2.8 \pm 0.2$ M.[41] As both reaction 8 and the interconversion between HNO_2 and NO^+ are reversible, so should be their combination, that is, reaction 12. This was convincingly demonstrated, and the equilibrium constant of reaction 12 was determined to be $K_{12} = (7.5 \pm 0.4) \times 10^{-4}$M.[41]

$$\mathrm{ONOOH} + \mathrm{H_2O} + \mathrm{H^+} \underset{k_{-12}}{\overset{k_{12}}{\rightleftharpoons}} \mathrm{HNO_2} + \mathrm{H_2O_2} + \mathrm{H^+} \qquad K_{12} = k_{12}/k_{-12} \quad (12)$$

By combining K_{12} with $\Delta_f G°(HNO_2) = -13.3$ kcal/mol,[64,65] $\Delta_f G°(H_2O_2) = -32.05$ kcal/mol,[64,66] and $\Delta_f G°(H_2O) = -56.68$ kcal/mol, one calculates $\Delta_f G°(ONOOH) = 7.1 \pm 0.2$ kcal/mol. Using $pK_a(ONOOH) = 6.6 \pm 0.1$, $\Delta_f G°(ONOO^-) = 16.1 \pm 0.3$ kcal/mol is obtained.

2.2.3. Gibbs' Energy of $ONOO^-$

A value of $\Delta_f G°(ONOO^-) = 16.3 \pm 0.2$ kcal/mol was obtained by treating the homolysis of $ONOO^-$ into $^\bullet NO$ and $O_2^{\bullet -}$ (equilibrium −6). The occurrence of

$$\mathrm{ONOO^-} \underset{k_{6}}{\overset{k_{-6}}{\rightleftharpoons}} {}^\bullet\mathrm{NO} + \mathrm{O_2}^{\bullet -} \qquad K_{-6} = k_{-6}/k_6 \quad (-6)$$

equilibrium −6 at pH > pK_a(ONOOH) was demonstrated,[36] and K_{-6} was obtained from the measurements of the rate constants for the forward and reverse reactions. The rate constant for the forward reaction was determined to be $k_{-6} = 0.020 \pm 0.003$ s^{-1} by use of either $C(NO_4)_2$[36,67] or methyl viologen radical[43] as efficient scavengers of $O_2^{\bullet -}$. Since $k_6 = (5 \pm 1) \times 10^9$ M^{-1} s^{-1},[17,43,52,53] one calculates $K_{-6} = (4 \pm 1) \times 10^{-12}$ M and $\Delta G°_{-6} = 15.7 \pm 0.2$ kcal/mol. Then, using the literature values of $\Delta_f G°(^\bullet NO) = 24.4$ kcal/mol[68] and $\Delta_f G°(O_2^{\bullet -}) = 7.6$ kcal/mol,[68] we obtain $\Delta_f G°(ONOO^-) = 16.3 \pm 0.2$ kcal/mol, which is in excellent agreement with 16.1 ± 0.3 kcal/mol, the latter having been determined by combining $\Delta_f G°$(ONOOH) with pK_a(ONOOH) (see section 2.2.2). Utilizing $\Delta_f G°(ONOOH) = 7.1 \pm 0.2$ kcal/mol, $\Delta_f G°(^\bullet NO_2) = 15.1$ kcal/mol,[68] and $\Delta_f G°(^\bullet OH) = 6.2$ kcal/mol,[68] we calculate $\Delta G°_{-4} = 14.1 \pm 0.2$ kcal/mol.

$$\mathrm{ONOOH} \underset{k_{4}}{\overset{k_{-4}}{\rightleftharpoons}} {}^\bullet\mathrm{NO_2} + {}^\bullet\mathrm{OH} \qquad K_{-4} = k_{-4}/k_4 \quad (-4)$$

The overall first-order rate constant for self-decomposition of ONOOH has been determined to be $k_0 = 1.25 \pm 0.05$ s^{-1} at 25 °C.[18,23,38] The yield of the radicals was found to be 28 ± 4%,[39,40] and therefore, $k_{-4} = 0.35 \pm 0.03$ s^{-1}. The rate constant of reaction 4 has been determined by pulse radiolysis to be $(5 \pm 1) \times 10^9$ M^{-1} s^{-1},[38] resulting in $K_{-4} = (7.0 \pm 2.5) \times 10^{-11}$ M and $\Delta G°_{-4} = 14.0 \pm 0.2$ kcal/mol. This value compares to $\Delta G°_{-4} = 14.1 \pm 0.2$ kcal/mol, which was determined utilizing the directly derived $\Delta_f G°(ONOOH) = 7.1 \pm 0.2$ kcal/mol. The above agreement fully bears out the free radical yield of homolysis to be ca. 30%.[39,40]

2.2.4. Homolysis of $ONOO^-$ into $^\bullet NO_2$ and $O^{\bullet -}$

It has been known for a long time that the measured rate constants for self-decomposition of peroxynitrite, k_d, at pH > 10 are substantially higher than those predicted on the assumption that ONOOH but not $ONOO^-$ decomposes into nitrate, that is, $k_d = k_0[H^+]/(K_a + [H^+])$.[13,14,69] The derived first-order rate constants for decomposition of peroxynitrite at pH 13 and 14 are very close, that is, 1.3×10^{-5} and 1.1×10^{-5} s^{-1}, respectively, and give the limiting rate constant $k_d \approx 1 \times 10^{-5}$ s^{-1} at 25 °C.[38] In addition, the decomposition of $ONOO^-$ at pH 14 was found to yield about 80% NO_3^-, 20% NO_2^-, and 10% O_2.[62] We, therefore, suggest that $ONOO^-$ decomposes at pH 14 via isomerization into NO_3^- (reaction 13) and homolysis along the O−O bond (eq 14). As was explained in detail elsewhere,[38] the generation of NO_2^- and O_2 at a ratio of 1:2 must occur via the consumption of two $ONOO^-$ ions for every homolysis in reaction 14. Hence, $k_d = k_{13} + 2k_{14}$, where $k_{13}/(k_{13} + 2k_{14}) \approx 0.8$. It follows that $k_{14}/k_{13} \approx 0.125$, $k_d \approx 11k_{14}$, and, hence, $k_{14} \approx 10^{-6}$ s^{-1}. Utilizing $\Delta_f G°(ONOO^-) = 16.3 \pm 0.4$ kcal/mol, $\Delta_f G°(^\bullet NO_2) = 15.1$ kcal/mol,[68] and $\Delta_f G°(O^{\bullet -}) = 22.4$ kcal/mol,[68] we obtain $K_{14} = (5.6 \pm 3.8) \times 10^{-16}$ M. The rate constant of reaction −14 was determined to be (3−4) × 10^9 M^{-1} s^{-1},[38] and therefore, $k_{14} = (2.0 \pm 1.9) \times 10^{-6}$ s^{-1}. This value is in very good agreement with the experimentally derived value and constitutes another strong piece of evidence for the homolysis reactions −4, −6, and 14 with the above-stated radical yields.

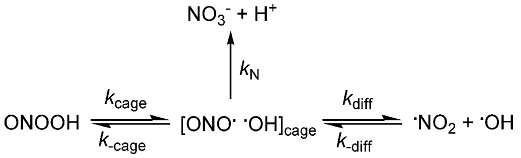
$$\mathrm{ONOO^-} \rightarrow \mathrm{NO_3^-} \quad (13)$$

$$\mathrm{ONOO^-} \underset{k_{-14}}{\overset{k_{14}}{\rightleftharpoons}} {}^\bullet\mathrm{NO_2} + \mathrm{O}^{\bullet -} \qquad K_{14} = k_{14}/k_{-14} \quad (14)$$

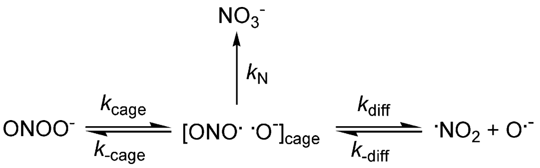
2.2.5. Description of Homolysis in Terms of the Radical Cage Model

The homolysis of chemical species into free radicals in aqueous solutions does not occur in one step, as in the gas phase at low pressures. Instead, the initial product of homolysis is a short-lived geminate pair with an average lifetime of less than 1 ns.[70,71] In principle, the geminate pair can diffuse out of the solvent cage into the bulk of the solution and become what is called "free" radicals, or it can collapse in the cage to form the starting material or some other end product. The detailed mechanisms of homolysis reactions −4 and 14 are presented in Schemes 2 and 3, respectively.

Scheme 2. Detailed Description of the Homolysis of ONOOH



Scheme 3. Detailed Description of the Homolysis of ONOO−



The radical pair in the cage can collapse to form ONOOH in Scheme 2 or $ONOO^-$ in Scheme 3 with $k_{-\mathrm{cage}}$ by forming a O−O bond and with k_N by forming a N−O bond, which results in the production of HNO_3 (which is immediately followed by dissociation into $NO_3^- + H^+$) in Scheme 2 or NO_3^- in Scheme 3.

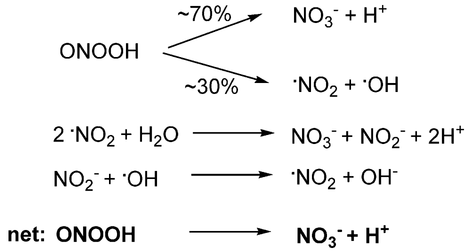
According to Scheme 2, the experimental decomposition rate constant, $k_0 = 1.25 \pm 0.05\ s^{-1}$ at 25 °C,[18,23,38] is expressed as $k_0 = k_{\mathrm{cage}}(k_{\mathrm{diff}} + k_N)/(k_{\mathrm{diff}} + k_{-\mathrm{cage}} + k_N)$. Since the radical yield is ca. 30%,[39,40] and since the combination reaction of $^{\bullet}NO_2$ with $^{\bullet}OH$ yields directly approximately equal amounts of ONOOH and $NO_3^- + H^+$,[38] it follows that $k_N/k_{\mathrm{diff}} \approx 2$ and $k_N/k_{-\mathrm{cage}} \approx 1$, respectively, and therefore $k_0 \approx 0.6k_{\mathrm{cage}}$.

2.2.6. Detailed Decomposition Mechanisms for ONOOH/ONOO−

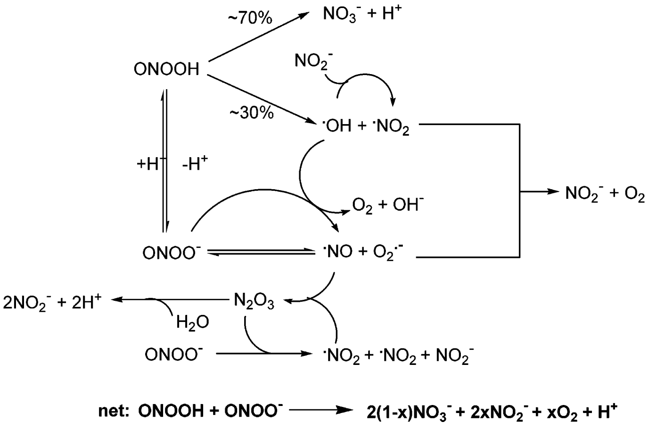
The decomposition of peroxynitrite in acidic solutions yields nitrate as a final product, but as the pH is raised, O_2 and nitrite in a 1:2 proportion are formed at the expense of nitrate, reaching ca. 40% O_2 at pH 9−10.[42,56,62,72,73] These results can be rationalized by assuming the homolysis reactions −4 and −6 and several other subsequent reactions of the reactive radicals produced in these reactions under different experimental conditions, as described in Schemes 4 and 5. It is worthy of note that the rate constants of all the invoked reactions have been measured separately.

Contrary to what has been suggested elsewhere,[61] the decomposition of ONOOH according to this reaction mechanism (Scheme 4) does not produce 85% nitrate and 15% nitrite, but almost 100% nitrate even in the absence of nitrite contamination. This is because $^{\bullet}OH$ reacts much faster, by about 2 orders of magnitude, with NO_2^- than with ONOOH. Consequently, $^{\bullet}OH$ will react with NO_2^- rather than consuming ONOOH, even when the former is only present in unavoidable trace amounts. As transpires from Scheme 4, small amounts of NO_2^-, whether present from the beginning or formed during the very early stages of ONOOH homolysis, will catalyze the overall conversion of ONOOH to $NO_3^- + H^+$ at pH 2−5. According to Scheme 1, the H^+-catalyzed decomposition of ONOOH will become the dominant mechanism at high acidities. The rate constants for the reaction of $^{\bullet}OH$ with $ONOO^-$ and NO_2^- have been determined to be 4.8×10^9 (ref 74) and $5.3 \times 10^9\ M^{-1}\ s^{-1}$,[38] respectively. As these rate constants are very similar, the reaction pattern of peroxynitrite will change when the pH approaches and exceeds pK_a(ONOOH) = 6.6, and the decomposition of peroxynitrite will form NO_2^- and O_2 at the expense of NO_3^-, as illustrated in Scheme 5.

Scheme 4. Decomposition of ONOOH



Scheme 5. Decomposition of ONOOH/ONOO−



The contribution of the reaction of $^{\bullet}OH$ with $ONOO^-$ depends on the ratio $[ONOO^-]/[NO_2^-]$. At the beginning of the decomposition process, this ratio will depend on the nitrite contamination present. However, given that NO_2^- is a decomposition product, $^{\bullet}OH$ will react preferentially with NO_2^- in the later stages of peroxynitrite decomposition. Scheme 5 also demonstrates the formation of the powerful electrophile N_2O_3. The rate constant of this species reacting with $ONOO^-$ is as high as $3.1 \times 10^8\ M^{-1}\ s^{-1}$,[75] and this reaction is in competition with the hydrolysis of N_2O_3, which is catalyzed by hydroxyl and phosphate ions.[76−79] Due to this competition, the decay rate of peroxynitrite as well as the product distribution become somewhat dependent on peroxynitrite con-

Table 2. Summary of the Reactions and Their Rate Constants Involved in the Decomposition Mechanism of Peroxynitrite at 25 °C

no.	reaction	*k*	ref
4	$^{\bullet}NO_2 + {}^{\bullet}OH \rightarrow ONOOH$	$(4.5 \pm 1.0) \times 10^9$ M^{-1} s^{-1}	38
−4	$ONOOH \rightarrow {}^{\bullet}NO_2 + {}^{\bullet}OH$	0.35 ± 0.03 s^{-1}	39, 40
	$ONOOH \rightarrow NO_3^- + H^+$	0.90 ± 0.05 s^{-1}	
	$ONOOH^a \rightarrow$ products	$k_0 = 1.25 \pm 0.05$ s^{-1}	18, 23, 38
5	$^{\bullet}NO + HO_2^{\bullet} \rightarrow ONOOH$	$(3.2 \pm 0.3) \times 10^9$ M^{-1} s^{-1}	17, 43, 52, 53
6	$^{\bullet}NO + O_2^{\bullet -} \rightarrow ONOO^-$	$(5 \pm 1) \times 10^9$ M^{-1} s^{-1}	17, 43, 52, 53
−6	$ONOO^- \rightarrow {}^{\bullet}NO + O_2^{\bullet -}$	0.020 ± 0.003 s^{-1}	36, 67
8	$ONOOH + H_2O + H^+ \rightarrow HNO_2 + H_2O_2$	6.3 ± 0.7 M^{-1} s^{-1}	41
9	$ONOOH + H^+ \rightarrow NO_3^- + 2\,H^+$	4.3 ± 0.1 M^{-1} s^{-1}	41
12	$ONOOH + H_2O \rightleftharpoons HNO_2 + H_2O_2$	$K_{12} = (7.5 \pm 0.4) \times 10^{-4}$ M	41
	$ONOO^- \rightarrow 0.8NO_3^- + 0.2NO_2^- + 0.1O_2$	$\approx 10^{-5}$ s^{-1}	38, 62
13	$ONOO^- \rightarrow NO_3^-$	$\approx 8 \times 10^{-6}$ s^{-1}	38, 62
14	$ONOO^- \rightarrow {}^{\bullet}NO_2 + O^{\bullet -}$	$\approx 10^{-6}$ s^{-1}	38, 62
−14	$^{\bullet}NO_2 + O^{\bullet -} \rightarrow ONOO^-$	$(3-4) \times 10^9$ M^{-1} s^{-1}	38
	$^{\bullet}OH + NO_2^- \rightarrow OH- + {}^{\bullet}NO_2$	5.3×10^9 M^{-1} s^{-1}	38
	$^{\bullet}OH + ONOO^- \rightarrow {}^{\bullet}NO + O_2 + OH^-$	4.8×10^9 M^{-1} s^{-1}	74
	$2{}^{\bullet}NO_2 + H_2O \rightarrow NO_2^- + NO_3^- + 2H^+$	$2k = 1.3 \times 10^8$ M^{-1} s^{-1}	80
	$^{\bullet}NO_2 + {}^{\bullet}NO \rightleftharpoons N_2O_3$	$k_f = 1.1 \times 10^9$ M^{-1} s^{-1}	81
		$k_b = 8.4 \times 10^4$ s^{-1}	
	$ONOO^- + N_2O_3 \rightarrow 2{}^{\bullet}NO_2 + NO_2^-$	3.1×10^8 M^{-1} s^{-1}	75
	$N_2O_3 + H_2O \rightarrow 2\,NO_2^- + 2H^+$	$2 \times 10^3 + 10^8[OH^-] + (6.4-9.4) \times 10^5$[phosphate] s^{-1}	76, 77, 82
	$ONOOH \rightleftharpoons ONOO^- + H^+$	$pK_a = 6.6 \pm 0.1$	16−18, 23

[a] ONOOH yields about 70% $NO_3^- + H^+$, while the remaining 30% gives $^{\bullet}NO_2 + {}^{\bullet}OH$ (reaction −4), which at pH < 5 forms eventually $NO_3^- + H^+$ (Scheme 4).

centration.[42,62,72] However, when the latter is sufficiently high, both quantities assume limiting concentration-independent values at any given pH. These findings are quantitatively reproduced by use of the measured rate constants of the reactions given in Scheme 5 and are ultimately predicated on the initial homolyses of ONOOH and $ONOO^-$.[42,62,72] The reactions and the rate constants involved in the decomposition mechanism of peroxynitrite are summarized in Table 2.

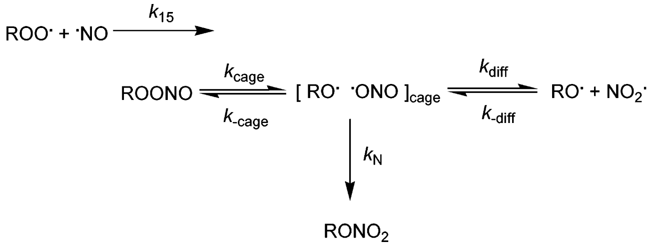
2.2.7. Opposing View on the Decomposition Mechanism for ONOOH/ONOO−

An alternative mechanism has also been suggested according to which the decomposition of peroxynitrite to O_2 and NO_2^- proceeds through a second-order reaction involving association of ONOOH and $ONOO^-$ to form a cyclic dimer that subsequently undergoes concerted bond rearrangement.[23,73] Such a mechanism has been described for the decomposition of organic and inorganic peroxides.[83−86] However, as was lucidly demonstrated,[42] this mechanism puts in contradiction the kinetic findings on one hand and the product yield on the other. Hence, the model is untenable. We do not doubt that such a reaction channel potentially exists even for peroxynitrite. However, it is apparently far too slow to compete with homolysis and is thus kinetically incompetent.•

2.3. Alkyl Peroxynitrites

$^{\bullet}NO$ reacts rapidly with alkyl peroxyl radicals ($ROO^{\bullet}$) to form alkyl peroxynitrites, ROONO. The rate constant for this reaction varied between 1.0×10^9 and 3.5×10^9 M^{-1} s^{-1}.[47,87] Recently, it was demonstrated that alkyl peroxynitrites do not accumulate but decompose rapidly via homolysis along the relatively weak O−O bond, initially forming a geminate pair (Scheme 7). Most of this pair collapses in the solvent cage to form alkyl nitrate, $RONO_2$, while about 11−16% diffuses out as free alkoxyl ($RO^{\bullet}$) and $^{\bullet}NO_2$ radicals; that is, $k_N/k_{diff} \approx 5-9$ (Scheme 6).[44,47]

Scheme 6. Detailed Description of the Formation and Homolysis of ROONO (Reaction 15)

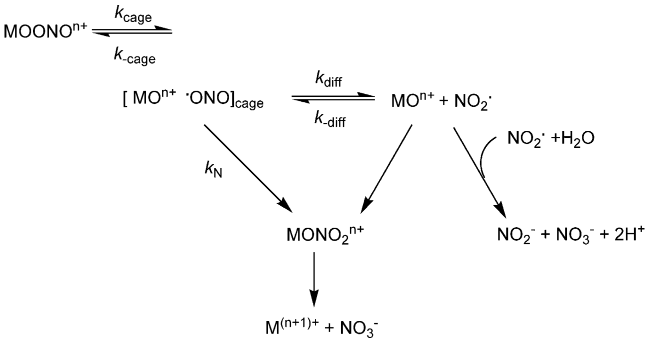


The mechanisms of decomposition of ROONO and HOONO are identical (Schemes 2 and 6). However, while in aqueous solutions the half-life of HOONO is about 1 s,[18,23,38] that of alkyl peroxynitrites is less than 1 μs.[44,47] This difference of about 6 orders of magnitude can be attributed to a decrease by about 6−8 kcal in the strength of the O−O bond upon replacing H by an alkyl group in a hydroperoxide.[88] Such a finding can be rationalized in terms of hyperconjugative stabilization of the alkoxyl radical as compared to the unstabilized hydroxyl radical.

2.4. Metal Peroxynitrites

The reaction between $^{\bullet}NO$ and oxyhemoglobin or oxymyoglobin is believed to be a major route for $^{\bullet}NO$ depletion in vivo.[89−91] This and analogous reactions with superoxometal complexes are fast ($k > 10^6$ M^{-1} s^{-1}) and ultimately produce nitrate and nitrite ions via an initial metal peroxynitrite intermediate.[92−95] Metal peroxynitrite species have also been proposed as intermediates formed via the reaction of peroxynitrite with metal complexes,[96] metalloporphyrins,[97−101] and hemeproteins.[102−104] Some of these metal complexes have been shown to catalyze the conversion of peroxynitrite to nitrate ions.[93,97,100,103,105,106] This catalysis process has been suggested to occur via the formation of a metal peroxynitrite adduct, which, in analogy with HOONO and ROONO, undergoes homolytic cleavage of the peroxo bond (Scheme 7).[94,95,97,100,102,103,105]

Scheme 7. Detailed Description of the Decomposition of Metal Peroxynitrites



The lifetimes of $MbFe^{III}OONO$, TMPyP−$Fe^{III}ONOO$, and $Cr_{aq}OONO^{2+}$ were reported to be about 4 ms,[93] 10 ms,[97] and <5 s,[95] respectively. Recently,[107] the O−O bond in $MbFe^{III}OONO$ was estimated to be weaker than that in alkylperoxynitrites, and while the homolysis of alkyl peroxynitrites is still slightly endergonic, the corresponding homolysis of $MbFe^{III}OONO$ was predicted to be exergonic. Given that the lifetime of alkyl peroxynitrites is below 1 μs,[44,47] the lifetime of $MbFe^{III}OONO$ is expected to be even shorter. In view of this, the previously[93] reported 4 ms lifetime for $MbFe^{III}OONO$ was almost certainly ascribed to the wrong intermediate. We believe that the intermediate with a lifetime of 4 ms[93] is the $MbFe^{III}ONO_2$ isomer. Indeed, when $MbFe^{III}ONO_2$ was produced directly via the reaction of $MbFe^{IV}{=}O$ with $^{\bullet}NO_2$, the same 4 ms lifetime was observed for it.[107]

2.5. Direct Reactions with Peroxynitrite

The oxidation by peroxynitrite can take place directly or indirectly via the highly reactive radicals formed during its decomposition. In fact, peroxynitrite oxidizes indirectly any substrate due to its decomposition into $^{\bullet}NO_2$ and $^{\bullet}OH$ radicals, as the latter radical is highly oxidizing. Peroxynitrous acid reacts directly with many substrates, and some few examples for the fastest reactions include sulfhydryls,[108] metal complexes,[109] ebselen,[110] iron[111] and manganese,[97,98,101] porphyrins, and heme proteins.[102,112−114] In the present review we shall focus on the nucleophilic addition of $ONOO^-$ to the C=O bond, which includes the important reaction with CO_2.

2.5.1. Reaction of Peroxynitrite with CO$_2$

Peroxynitrite was found to be unstable in the presence of carbonate buffer.[115] The biological implications of this observation were recognized only after two decades, when it was found that bicarbonate inhibited *Escherichia coli* killing by peroxynitrite.[116,117] Later, it was shown that CO_2 reacts with $ONOO^-$ relatively fast; that is, $k_{16} = (2.9 \pm 0.3) \times 10^4$ M^{-1} s^{-1} at 24 °C, apparently forming an adduct whose composition is believed to be $ONOOC(O)O^-$.[118]

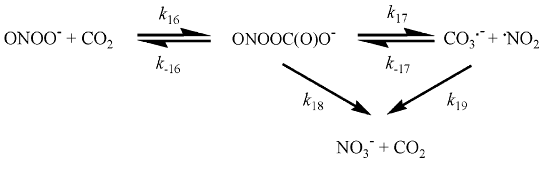
$$ONOO^- + CO_2 \rightarrow ONOOC(O)O^- \qquad (16)$$

The concentration of CO_2 in vivo is relatively high due to high levels of bicarbonate in intracellular (12 mM) and interstitial fluids (30 mM).[119] This suggests that the reaction of peroxynitrite with CO_2 must be the predominant pathway for peroxynitrite disappearance in biological systems. It was found that CO_2 reacts with $ONOO^-$ to yield NO_3^- as a final product[120,121] and that $ONOOC(O)O^-$ participates in oxidation and nitration processes.[40,50,120−130] In this section we shall review the *experimental* evidence in support of the homolysis of $ONOOC(O)O^-$ into ca. 33% $^{\bullet}NO_2$ and $CO_3^{\bullet-}$ in the bulk of the solution.

2.5.1.1. Direct Detection of $CO_3^{\bullet-}$. The carbonate ion radical was directly detected by its EPR parameters in flow mixtures of peroxynitrite with bicarbonate at pH 6−9[131] or upon mixing alkaline peroxynitrite with CO_2-saturated solution in the EPR cavity.[132] However, the estimation of the radical yield varied from a few percent[132] to 35%.[131] The carbonate ion radical was also directly detected upon mixing peroxynitrite with an excess of CO_2 in alkaline solutions, and its yield was estimated to be about 33% using $\epsilon_{600} = 1860$ M^{-1} cm^{-1}.[133]

2.5.1.2. Oxidation Yield. In the presence of excess CO_2, peroxynitrite oxidizes or nitrates a large variety of substrates, but the oxidation or nitration yields do not exceed ca. 33% of added peroxynitrite. Equally important is the observation that the decay of $ONOO^-$ in the presence of excess CO_2 and the formation of the oxidized or nitrated substrates occur at the same rate.[40,50,120,121,123,126,127,133] These observations demonstrate that $ONOOC(O)O^-$ does not accumulate but decomposes rapidly to form about 33% $^{\bullet}NO_2$ and $CO_3^{\bullet-}$ radicals, which in the absence of potential reductants proceed to yield nitrate. The proposed radical model is presented in Scheme 8, where $k_{18}/k_{17} \approx 2$.

Scheme 8. Decomposition of Peroxynitrite in the Presence of an Excess of CO$_2$ (Reactions 17−19)



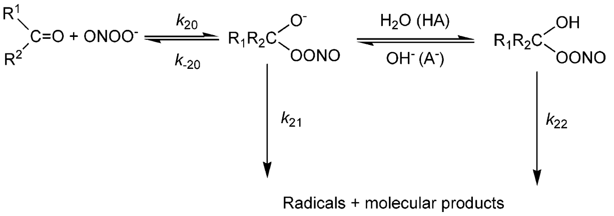
2.5.1.3. Viscosity Effect. It has been well established that the rate of diffusion of radicals out of a solvent cage decreases with increasing solvent viscosity.[134] Therefore, if a caged radical pair is formed, changes in the viscosity of the solvent should affect the product yields and the rate constant for decomposition of the free radical initiators. Indeed, $ONOO^-$ in the presence of excess CO_2 oxidized $Fe(CN)_6^{4-}$ or $ABTS^{2-}$, and the yields of $Fe(CN)_6^{3-}$ or $ABTS^{\bullet-}$, which were 30−33% of added peroxynitrite, decreased upon increasing the concentration of added glycerol.[135] Furthermore, the oxidation yield of $ABTS^{•-}$ was independent of $[ABTS^{2-}]_0$ and was within experimental error identical to that of $Fe(CN)_6^{3-}$, when the same amount of glycerol was added.[135] These results demonstrate that the decrease in the oxidation yields with increasing glycerol concentration does not result from a competition between glycerol and $ABTS^{2-}$ or $Fe(CN)_6^{4-}$ for the oxidizing radicals but is due to the increase in the viscosity. This observation suggests the formation of oxidizing radicals, that is, $CO_3^{•-}$ and $^•NO_2$, in a water cage, where only about 33% of the radicals escape from the water cage to the bulk of the solution.

2.5.1.4. Thermodynamics of the Reaction of $ONOO^-$ with CO_2. The equilibrium constant K_{16} was estimated by assuming that the equilibrium constant for formation of an adduct between the C═O double bond of a certain compound and a hydroperoxide anion varies linearly with the acid dissociation constant of the hydroperoxide. Specifically, if the two hydroperoxide anions HOO^- ($pK_a(HOOH) = 11.7$) and $ONOO^-$ ($pK_a(ONOOH) = 6.6$) add to CO_2, and with the dissociation constant of $HOOC(O)O^-$ being $6.5 \times 10^4\ M^{-1}$,[45,46,136] one calculates $K_{16} \approx 0.5\ M^{-1}$. A somewhat lower value, that is, $K_{16} = 0.02\ M^{-1}$, was estimated using a linear relationship between the equilibrium constant for formation of alkyl monocarbonates ($ROC(O)O^-$) in the reaction of alkoxides (RO^-) with CO_2 and the dissociation constant of the respective alcohol (ROH).[45] Utilizing $\Delta_f G°(ONOO^-) = 16.3 \pm 0.4$ kcal/mol, $\Delta_f G°(CO_2) = -92.2$ kcal/mol, $\Delta_f G°(CO_3^{•-}) = -89.5$ kcal/mol,[137] and $\Delta_f G°(^•NO_2) = 15.1$ kcal/mol,[68] one calculates $(\Delta G°_{16} + \Delta G°_{17}) = -RT\ \ln(K_{16}K_{17}) = 1.5$ kcal/mol, $K_{16}K_{17} = 0.08$, and $K_{17} \approx 0.16$ M. The relatively low value of $\Delta G°_{16} \approx 1.1$ kcal/mol indicates that the O−O bond in $ONOOC(O)O^-$ is very weak. Theoretical calculations were also in agreement with this conclusion.[138] Hence, $ONOOC(O)O^-$ should be a very short-lived adduct, and from experimental data[139] coupled with reasonable assumptions, the rate constant for its decay was estimated to lie between 10^7 and $10^9\ s^{-1}$.[45,46] Clearly, with a lifetime below ca. 0.1 μs, $ONOOC(O)O^-$ should have no time to react with any biological matter prior to homolyzing into $CO_3^{•-}$ and $^•NO_2$ radicals. Consequently, any biological damage that occurs in the simultaneous presence of $ONOO^-$ and CO_2 must be ascribed to the latter two radical species.

2.5.2. Reaction of Peroxynitrite with Carbonyls

The addition of $ONOO^-$ to carbonyl compounds complies with the well-established pattern of nucleophilic addition. The latter has been extensively explored in several processes, for example, ester hydrolysis, and the whole impressive armament of physical organic chemistry has been brought to bear on these biologically vital reactions.[140,141] In Scheme 9, the various reactions are given in the case where $ONOO^-$ is the nucleophile. The only feature in Scheme 9 which is unique to $ONOO^-$ as the nucleophile is that its adduct (unlike e.g., the OH^- adduct) can undergo homolysis reaction 21 or 22.

Scheme 9. Nucleophilic Addition of ONOO− to Carbonyls (Reactions 20−22)



The process is initiated by fast equilibration between the reactants and the corresponding tetrahedral adduct anion, with the equilibrium being strongly shifted to the reactant side. The adduct anion also undergoes fast protonation by water and added buffers.[44,142] The rate of nucleophilic addition of $ONOO^-$ to carbonyls is a much slower process compared to addition to CO_2.[40,44,142,143] In particular, addition of $ONOO^-$ to amides, which potentially could have biological relevance, appears not to occur.[144] Thus, overall, addition to carbonyls has probably negligible biological importance.

3. Peroxynitrate

3.1. Formation of Peroxynitrate in Aqueous Solutions

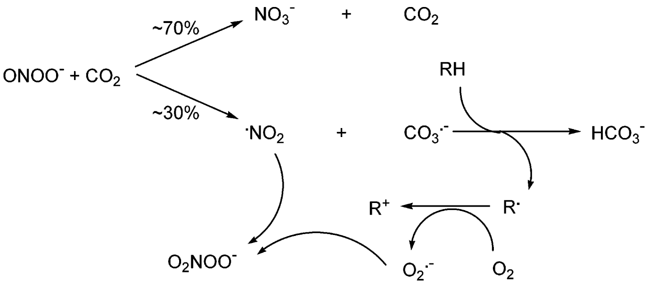
Peroxynitrate (O_2NOOH/O_2NOO^-) is formed in aqueous solution through the very fast recombination of $HO_2^•/O_2^{•-}$ with $^•NO_2$ (reactions 23 and 24).[16,20,145]

$$HO_2^• + {}^•NO_2 \underset{k_{-23}}{\overset{k_{23}}{\rightleftharpoons}} O_2NOOH \qquad K_{23} = k_{23}/k_{-23} \tag{23}$$

$$O_2^{•-} + {}^•NO_2 \underset{k_{-24}}{\overset{k_{24}}{\rightleftharpoons}} O_2NOO^- \qquad K_{24} = k_{24}/k_{-24} \tag{24}$$

The rate constants for reactions 23 and 24 have been determined by pulse radiolysis to be $(1.8 \pm 0.2) \times 10^9$ and $(4.5 \pm 1.0) \times 10^9\ M^{-1}\ s^{-1}$, respectively.[16] Most significantly, peroxynitrate can be formed during the decomposition of peroxynitrite under physiological conditions.[40,50,146,147] Reaction Scheme 10 demonstrates the formation of peroxynitrate during the decomposition of peroxynitrite in the presence of CO_2, which is considered to be the main sink for peroxynitrite under physiological conditions.[118]

Scheme 10. Conversion of Peroxynitrite into Peroxynitrate under Physiological Conditions



3.2. Mechanism and Thermochemistry of Peroxynitrate Decomposition in Aqueous Solutions

In this section, we shall present the *experimental* evidence in support of the dissociation of O_2NOOH

into $HO_2^{\bullet}$ and $^{\bullet}NO_2$ as well as the derived reaction schemes for the decomposition of peroxynitrate under different experimental conditions.

3.2.1. Activation Parameters

Peroxynitric acid ($pK_a = 5.9 \pm 0.1$)[16,20] is a relatively stable species in aqueous solutions ($\tau_{1/2} = 17-70$ min),[16,19,148] whereas the anion decomposes relatively fast to yield at pH > 5 only nitrite and oxygen.[149] The activation parameters for the decomposition of O_2NOO^- into NO_2^- and O_2 were determined to be $A = 3 \times 10^{18}$ s^{-1},[150] $E_a = 25.2 \pm 0.4$ kcal/mol,[150] and $\Delta V^* = 6.7 \pm 0.7$ cm^3/mol.[58] The activation parameters are summarized in Table 3.

Table 3. Activation Parameters for Peroxynitrate at 25 °C

	E_a, kcal/mol	A, s^{-1}	ΔV^*, cm^3/mol
$O_2NOO^- \rightarrow NO_2^- + O_2$	25.2[150]	3×10^{18} (ref 150)	6.7 ± 0.7[58]
$O_2NOOH \rightarrow HO_2^{\bullet} + {}^{\bullet}NO_2$	26.3[19]	5.27×10^{16} (ref 19)	nd[a]

[a] nd = not determined.

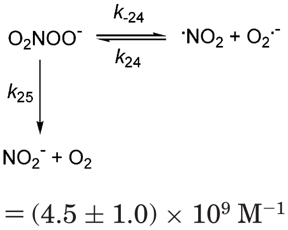
3.2.2. Mechanism of O_2NOOH Decomposition below pH 3.5

The rate constant of O_2NOOH decomposition at pH < 3.5 in the presence of cupric ions[19] or $C(NO_2)_4$[21] has been determined to be (0.026 ± 0.003) s^{-1}, and the yield of $C(NO_3)_3^-$ approached 100% of added peroxynitrate.[21] These results imply, as previously suggested,[149,151] that decay of O_2NOOH occurs via equilibrium −23, with $K_{-23} = 1.4 \times 10^{-11}$ M. This is followed by dismutation of $HO_2^{\bullet}$ and hydrolysis of $^{\bullet}NO_2$. The value of $\Delta_fG°(O_2NOOH)$ was reported as 1.3 ± 0.4 kcal/mol.[19]

3.2.3. Mechanism of O_2NOO— Decomposition above pH ca. 6.7

The rate constant for decomposition of O_2NOO^- at pH > 6.7 increased upon addition of excess $C(NO_2)_4$ from 1.35 ± 0.03 to 2.4 ± 0.2 s^{-1} at 25 °C. The latter value was independent of $[C(NO_2)_4]_0$, and the yield of $C(NO_3)_3^-$, which is formed via the reduction of $C(NO_2)_4$ by superoxide, was $47 \pm 5\%$ of peroxynitrate.[21] The data show that O_2NOO^- dissociates into $^{\bullet}NO_2$ and $O_2^{\bullet-}$ and that the contribution of this reaction to the decomposition of O_2NOO^- is about 50%; that is, $k_{-24}/k_{25} \approx 1$ (Scheme 11).

Scheme 11. Decomposition of O_2NOO— (Reaction 25)



Utilizing $k_{24} = (4.5 \pm 1.0) \times 10^9$ M^{-1} s^{-1} [16] and $k_{-24} = 1.05 \pm 0.23$ s^{-1} [21], we calculate $K_{24} = (4.3 \pm 1.6) \times 10^9$ M^{-1} and $\Delta G°_{24} = -13.1 \pm 0.3$ kcal/mol. Hence, using $\Delta_fG°({}^{\bullet}NO_2) = 15.1$ kcal/mol[68] and $\Delta_fG°(O_2^{\bullet-}) = 7.6$ kcal/mol,[68] $\Delta_fG°(O_2NOO^-) = 9.6 \pm 0.3$ kcal/mol is obtained. As $\Delta_fG°(O_2NOOH) = 1.3 \pm 0.4$ kcal/mol,[19] we immediately obtain $pK_a(O_2NOOH) = 6.1 \pm 0.3$, which is in excellent agreement with the experimental value of 5.9 ± 0.1.[16,20] The reactions and their rate constants involved in the decomposition mechanism of peroxynitrate are summarized in Table 4. The Gibbs energies of peroxynitrate and peroxynitrite are summarized in Table 5.

Table 4. Summary of the Reactions and Their Rate Constants Involved in the Decomposition Mechanism of Peroxynitrate at 25 °C

no.	reaction	k	ref
23	$^{\bullet}NO_2 + HO_2^{\bullet} \rightarrow O_2NOOH$	$(1.8 \pm 0.2) \times 10^9$ M^{-1} s^{-1}	16
−23	$O_2NOOH \rightarrow {}^{\bullet}NO_2 + HO_2^{\bullet}$	0.026 ± 0.003 s^{-1}	19, 21
24	$^{\bullet}NO_2 + O_2^{\bullet-} \rightarrow O_2NOO^-$	$(4.5 \pm 1.0) \times 10^9$ M^{-1} s^{-1}	16
−24	$O_2NOO^- \rightarrow {}^{\bullet}NO_2 + O_2^{\bullet-}$	1.05 ± 0.23 s^{-1}	21
25	$O_2NOO^- \rightarrow NO_2^- + O_2$	1.35 ± 0.03 s^{-1}	21
	$O_2NOOH \rightleftharpoons O_2NOO^- + H^+$	$pK_a = 5.9 \pm 0.1$	16, 20

Table 5. Thermodynamic Parameters of ONOOH/ONOO− and O_2NOOH/O_2NOO^- in Aqueous Solution at 25 °C

species	$\Delta_fG°$ (kcal/mol)	$\Delta_fH°$ (kcal/mol)	$S°$ [a] (cal/mol·K)
ONOOH	7.1 ± 0.2[41]	-14.3 ± 1.5[38]	40 ± 5
$ONOO^-$	16.3 ± 0.4[38]	-10 ± 1[152,153]	24 ± 4
O_2NOOH	1.3 ± 0.4[19]	-26.5 ± 0.5[19]	44 ± 2
O_2NOO^-	9.6 ± 0.3 (this work)		

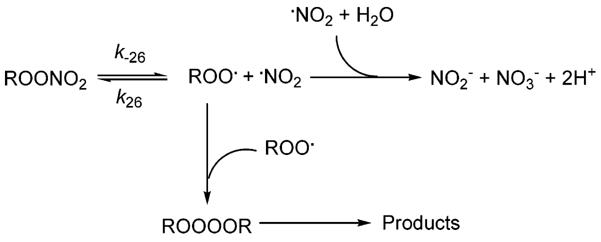
[a] The $S°$ values were calculated using the $\Delta_fG°$ and $\Delta_fH°$ values tabulated in Table 5, as well as the $S°$ values for the appropriate elements.

The question whether reaction 25 yields $^1O_2$ in a concerted reaction or yields $^3O_2$ as a cage product formed upon homolysis followed by electron transfer cannot be answered with absolute certainty. The rate constant of quenching of $^1O_2$ by NO_2^- was determined to be 3.1×10^6 M^{-1} s^{-1}, and the immediate product of the quenching reaction was suggested to be O_2NOO^-.[154] Coupled with $\Delta_fG°(O_2NOO^-) = 9.6$ kcal/mol, $\Delta_fG°({}^1O_2) = 26.8$ kcal/mol,[68] and $\Delta_fG°(NO_2^-) = -8.9$ kcal/mol,[155] the reverse rate constant is predicted to be ca. 2.5 s^{-1}, which, considering the uncertainties in the employed quantities, is close to $k_{25} = 1.35$ s^{-1}. We are aware of the fact that the extremely high pre-exponential factor of reaction 25, $A = 3 \times 10^{18}$ s^{-1}, as well as its positive activation volume, would appear more characteristic of a homolysis reaction. However, when ions are involved in a dissociation reaction, differences in specific solvation can give rise to unusual activation parameters. Hence, unlike in cases with only neutral species involved, these parameters may not be safe indicators of reaction type. In view of the above, we opt for reaction 25 being concerted and to produce predominantly $^1O_2$. This conclusion is supported by quantum chemical calculations.[156] The formation of $^1O_2$ has yet to be confirmed or disproved experimentally, though some indirect experiments have demonstrated its formation during the decomposition of peroxynitrite in the presence of H_2O_2.[157,158] A plausible explanation for the latter observation is that the reaction of peroxynitrite with H_2O_2 forms peroxynitrate,[40] which decomposes to yield nitrite and $^1O_2$.

3.3. Alkyl Peroxynitrates

In the gas phase there exists a substantial body of data related to the kinetics of formation and decomposition of alkyl peroxynitrates.[9−11,159,160] Recently, the reaction of $^{\bullet}NO_2$ with alkyl peroxyl radicals, such as c-$C_5H_9OO^{\bullet}$ (cyclopentanyl peroxyl radical), $CH_3OO^{\bullet}$, and $(CH_3)_2C(OH)CH_2OO^{\bullet}$, was studied in aqueous solutions.[47] It has been shown that $ROO^{\bullet}$ readily reacts with $^{\bullet}NO_2$ ($k_{26} > 7 \times 10^8$ M^{-1} s^{-1}) to form relatively long-lived peroxynitrate species, which have no appreciable absorption above 260 nm.[47] It has also been established that, just as in the gas phase, the rate-determining step in the decomposition of a particular $ROONO_2$ is the dissociation into $^{\bullet}NO_2$ and $ROO^{\bullet}$.[44,47] The evidence was provided by the observation that the rate of formation of $O_2^{\bullet -}$, sometimes produced during the decay of $ROO^{\bullet}$, was similar to the rate of $ROONO_2$ decomposition.[44,47] We conclude that $ROONO_2$ decomposes via dissociation into $ROO^{\bullet}$ and $^{\bullet}NO_2$ followed by the decomposition of $ROO^{\bullet}$ and hydrolysis of $^{\bullet}NO_2$ (Scheme 12).

Scheme 12. Mechanism of $ROONO_2$ Decomposition (Reaction 26)



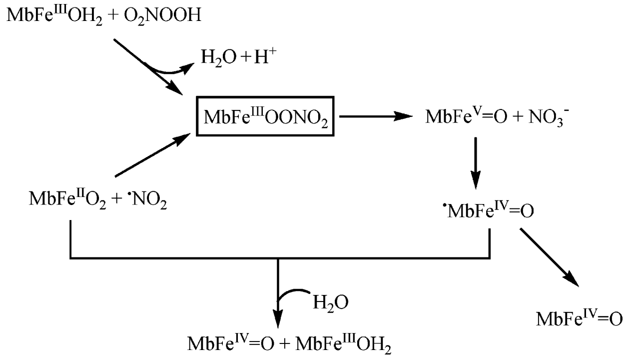
This mechanism is similar to that described for $HOONO_2$, which decomposes slowly in acidic solutions via dissociation into $HO_2^{\bullet}$ and $^{\bullet}NO_2$ followed by dismutation of $HO_2^{\bullet}$ and hydrolysis of $^{\bullet}NO_2$.[149,151] As was pointed out,[47] in the gas phase the rate of the dissociation of a peroxynitrate into $ROO^{\bullet}$ and $^{\bullet}NO_2$ closely parallels its equilibrium constant for dissociation. This appears to be the case also in aqueous solutions.[47]

3.4. Metal Peroxynitrates

Metal peroxynitrates have been proposed as intermediates in the reaction of $^{\bullet}NO_2$ with $Cr_{aq}OO^{2+}$ (refs 161 and 162) and L^2RhOO^{2+}.[94] The reaction between $Cr_{aq}OO^{2+}$ and $^{\bullet}NO_2$ did not consume significant amounts of $Cr_{aq}OO^{2+}$, suggesting that $Cr_{aq}OONO_2^{2+}$ decomposes mainly via homolytic cleavage of the N−O bond and hydrolysis of $^{\bullet}NO_2$.[161] The rate constant for this process, 172 s^{-1},[161] is several orders of magnitude greater than that for $HOONO_2$, 0.026 s^{-1}.[19,21]

Recently, the short-lived $MbFe^{III}OONO_2$ was suggested to form as an intermediate in the reaction between $MbFe^{II}O_2$ and $^{\bullet}NO_2$ or between $MbFe^{III}OH_2$ and O_2NOOH.[107] $Cr_{aq}OONO_2^{2+}$ and $MbFe^{III}OONO_2$ are akin to the respective peroxo adducts $Cr_{aq}OOH^{2+}$ and $MbFe^{III}OOH$, which are formed as intermediates via the reduction of $Cr_{aq}OO^{2+}$ by one electron donors[163] or through the reaction of $MbFe^{III}OH_2$ with H_2O_2,[164] respectively. However, $Cr_{aq}OOH^{2+}$ is stable with respect to heterolysis of the O−O bond.[163] In view of this stability, the decay of $Cr_{aq}OONO_2^{2+}$ solely by way of O−N homolysis is understood. By contrast, $MbFe^{III}OOH$ decomposes fast into a ferryl having the radical site on the globin, $^{\bullet}MbFe^{IV}{=}O$.[164−168] On the basis of this observation, it has been proposed that $MbFe^{III}OONO_2$ also decomposes via heterolysis of the O−O bond to form $MbFe^{V}{=}O$ and NO_3^-. Subsequently, $MbFe^{V}{=}O$ rapidly transforms into $^{\bullet}MbFe^{IV}{=}O$, which can either decompose to $MbFe^{IV}{=}O$ or oxidize $MbFe^{II}O_2$ (Scheme 13).[107]

Scheme 13. Mechanism of Formation and Decomposition of $MbFe^{III}OONO_2$



3.5. Direct and Indirect Reactions with Peroxynitrate

In principle, the oxidation by peroxynitrate can take place directly or indirectly via $^{\bullet}NO_2$ and $HO_2^{\bullet}/O_2^{\bullet -}$, which are in equilibrium with O_2NOOH/O_2NOO^-. Hence, peroxynitrate is less aggressive than peroxynitrite, simply because superoxide is less reactive than $^{\bullet}OH$. Peroxynitric acid was shown to react directly with halides[20,148,149,169] and with HNO_2[16] and much faster with $MbFe^{III}OH_2$.[107] The rate constant for the oxidation of I^- by O_2NOO^- is significantly lower than that for the oxidation by O_2NOOH, that is, 56 and 840 ± 50 M^{-1} s^{-1}, respectively.[20] While the rate constant for the oxidation of I^- by ONOOH ($(1.15 \pm 0.05) \times 10^4$ M^{-1} s^{-1})[109] is considerably higher than that for the oxidation by O_2NOOH (840 ± 50 M^{-1} s^{-1}),[20] the addition of O_2NOO^- to $MbFe^{III}OH_2$ ($(4.6 \pm 0.3) \times 10^4$)[107] is somewhat faster as compared to addition of $ONOO^-$ ($(1.03 \pm 0.3) \times 10^4$ M^{-1} s^{-1}).[102] There are only a few examples of indirect oxidation by peroxynitrate, for example, with NADH,[20] tetranitromethane,[21] $Cu^{2+}(aq)$,[19,21] $Fe(CN)_6^{4-}$,[20,21] and halogens.[169] Of particular interest was the finding that O_2NOOH acted not only as an oxidant but also as a reductant, as it was shown to reduce halogens through $HO_2^{\bullet}$, the radical present in equilibrium with O_2NOOH.[169]

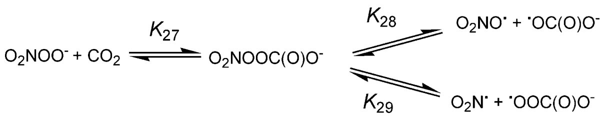
3.5.1. Peroxynitrate in the Presence of CO_2

Under physiological conditions peroxynitrate is predominantly in its anionic form, as $pK_a(ONOOH) = 5.9$.[16,20] Hence, the toxicity of this peroxide might be governed by the product of the reaction of O_2NOO^- with CO_2, which most probably would be

$O_2NOOC(O)O^-$. However, peroxynitrate was shown to be stable in the presence of bicarbonate at physiological pH.[40,50]

A conceivable mechanism for the reaction of O_2NOO^- with CO_2 is given in Scheme 14. It is expected that the half-life of $O_2NOOC(O)O^-$ would be significantly longer than that of $ONOOC(O)O^-$. This expectation is based on O_2NOOH being relatively stable, showing no tendency to homolyze along the O−O bond. $O_2NOOC(O)O^-$ could in principle undergo either homolysis of the O−O bond, forming ${}^{\bullet}NO_3$ and $CO_3^{\bullet-}$ (reaction 28), or homolysis of the N−O bonds, forming ${}^{\bullet}NO_2$ and $CO_4^{\bullet-}$ (reaction 29).

Scheme 14. Reaction of O_2NOO^- with CO_2 (Reactions 27−29)



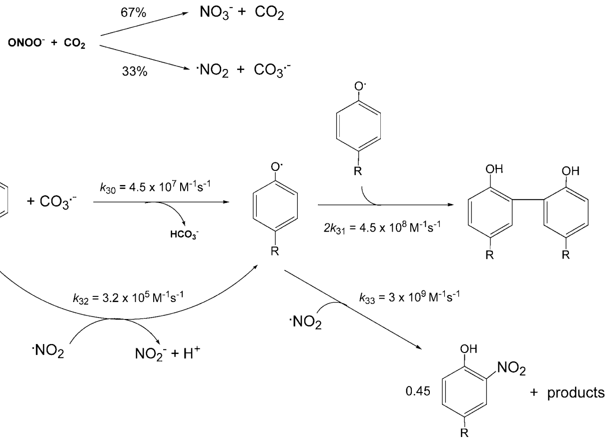
The equilibrium constant $K_{27} \approx 0.1\ M^{-1}$ was estimated[46] in a fashion similar to that used to find $K_{16} \approx 0.5\ M^{-1}$. The closeness of the values parallels the similar acidities of the two hydroperoxides. However, both equilibria 28 and 29 were estimated to be extremely unfavorable.[46] Hence, both homolysis pathways should be blocked in practice. We therefore concluded that, at realistic concentrations, the presence of CO_2 cannot modify the natural decay rate of peroxynitrate,[46] which is about $1\ s^{-1}$ at room temperature and physiological pH. The unfavorable energetics of homolysis reactions 28 and 29 were supported by quantum chemical calculations.[156]

4. Biological Implications of the Radical Model

The importance of the coupling of ${}^{\bullet}NO$ with $O_2^{\bullet-}$ to yield $ONOO^-$ in biological systems was first suggested by Beckman et al.,[24] and this reaction is currently accepted as the main biological source of peroxynitrite. The reactivity of peroxynitrite toward biological molecules and its very high toxicity toward cells are assumed to be the potential cause of a number of diseases.[25−28,170−172] Under physiological conditions, where high concentrations of bicarbonate prevail, most peroxynitrite is scavenged by CO_2, forming ${}^{\bullet}NO_2$ and $CO_3^{\bullet-}$, which are highly nitrating and oxidizing radicals. However, under physiological conditions the precursors of peroxynitrite, namely ${}^{\bullet}NO$ and $O_2^{\bullet-}$ radicals, are generated by low and not necessarily equal fluxes. The homolysis of $ONOOC(O)O^-$ into ${}^{\bullet}NO_2$ and $CO_3^{\bullet-}$ implies that a continuous generation of peroxynitrite should have an enormous effect on its reactivity, especially when the toxic products are formed via a radical−radical reaction. We shall demonstrate this effect on the nitration of tyrosine, which is considered as a biomarker for ${}^{\bullet}NO$-dependent oxidative stress in vivo.[25−27] However, despite the capacity of peroxynitrite to mediate tyrosine nitration in vitro,[121,123,173,174] its role in nitration in vivo has been questioned,[175] and alternative pathways, including nitrite/H_2O_2/hemeperoxidase[176] or nitrite/H_2O_2/hemeproteins[104,177−179] or transition metal-dependent mechanisms,[180] have been proposed.

The mechanism of the nitration and oxidation of tyrosine by authentic peroxynitrite in the presence of excess CO_2 at pH 7.5 is given in Scheme 15.[174] This mechanism was unequivocally proven by CIDNP experiments.[33,181,182] All the rate constants in this reaction scheme are known,[183−185] and under such conditions the dimerization of $CO_3^{\bullet-}$ and hydrolysis of ${}^{\bullet}NO_2$ are relatively slow and can be ignored. As about 33% of peroxynitrite yields $CO_3^{\bullet-}$ and ${}^{\bullet}NO_2$, and since the reaction of $CO_3^{\bullet-}$ with tyrosine is relatively fast, about 33% of peroxynitrite forms tyrosyl radical ($TyrO^{\bullet}$) and ${}^{\bullet}NO_2$ even in the presence of relatively low concentrations of tyrosine. Hence, the initial concentrations of these radicals are relatively high, and therefore, most of them will cross-recombine to yield coupling products. It was shown that 3-nitrotyrosine makes up about 45% of the coupling products.[174] Hence, the nitration yield in the presence of 1−2 mM tyrosine should be about 15%, in agreement with experimental results.[123,174,186]

Scheme 15. Mechanism of Nitration and Oxidation of Tyrosine by Authentic Peroxynitrite in the Presence of an Excess of CO_2 at pH 7.5 (Reactions 30−33)



However, the yield of dityrosine was found to increase at the expense of 3-nitrotyrosine upon substantial lowering of the initial peroxynitrite concentration, or when peroxynitrite was generated continuously.[174,187] Under such conditions, the lowered radical flux causes a decrease in the *absolute steady-state concentration of all radicals* in the system. As a result, any potential reaction between a radical and a nonradical substrate will be favored compared to radical−radical reactions. Specifically, in the present system, reaction 32 (Scheme 15), that is, the reaction of tyrosine with ${}^{\bullet}NO_2$, the only remaining radical with a moderate reactivity toward tyrosine, will come into efficient competition with reaction 33 (Scheme 15), the reaction coupling ${}^{\bullet}NO_2$ with the tyrosyl radical. Hence, *the steady-state concentration of ${}^{\bullet}NO_2$ will decrease relative to the that of the tyrosyl radical*. The same efficient competition between reactions 32 and 33 takes place if at a constant radical flux the concentration of tyrosine is increased. The overall outcome will be that the relative rate of the radical coupling reaction 31 yielding dityrosine will increase dramatically at the expense of the radical coupling reaction 33, yielding

3-nitrotyrosine. To conclude, the nitration yield will be dependent on the flux of peroxynitrite, that is, the radical flux, as well as on the concentration of tyrosine. This is demonstrated in Figure 1.

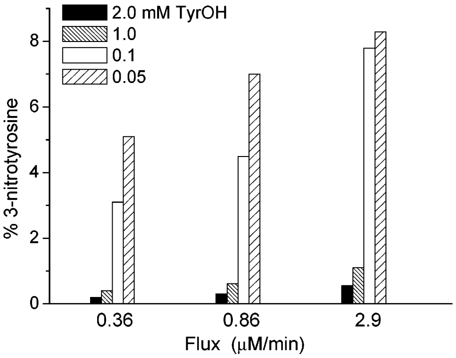


Figure 1. Reaction at equal fluxes of $^\bullet$NO and $O_2^{\bullet-}$ with tyrosine in the presence of 0.38 mM CO_2 at pH 7.5. The nitration yields in the presence of 2 mM tyrosine are experimental data,[174] and the others were simulated assuming the model proposed in Scheme 15.

Upon excess production of $O_2^{\bullet-}$, the steady-state concentration of TyrO$^\bullet$ is significantly reduced due to reaction 34. Thus, while reaction 32 is still the

$$\mathrm{TyrO^\bullet + O_2^{\bullet-} \rightarrow products} \qquad k_{34} = 1.5 \times 10^9\ \mathrm{M^{-1}\ s^{-1}}\ \text{(ref 185)} \quad (34)$$

major consumption route of $^\bullet NO_2$, reaction 33 is suppressed in favor of reaction 34, and nitration is completely inhibited in the presence of excess generation of $O_2^{\bullet-}$. At excess production of $^\bullet$NO, the steady-state concentration of TyrO$^\bullet$ is unaffected due to the reversible binding of $^\bullet$NO to TyrO$^\bullet$.[174] However, the steady-state concentration of $^\bullet NO_2$ is reduced due to its reaction with $^\bullet$NO to form N_2O_3, which hydrolyzes fast under physiological conditions.[76-79] The simulated nitration yields are given in Figure 2 at various fluxes of $^\bullet$NO and $O_2^{\bullet-}$ and at different tyrosine concentrations. These simulation results demonstrate that the maximum nitration yield is obtained at equal fluxes of these radicals.

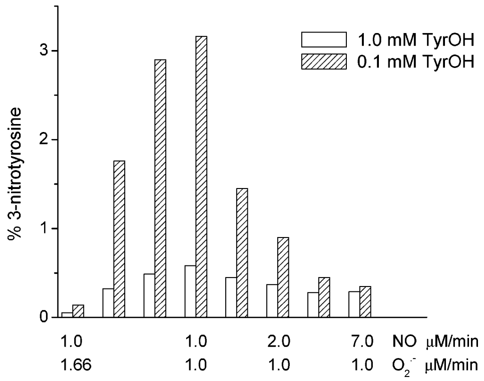


Figure 2. Reaction of different fluxes of $^\bullet$NO and $O_2^{\bullet-}$ with tyrosine in the presence of 1 mM CO_2 at pH 7.5. The nitration yields were simulated assuming the model proposed in Scheme 15.

Figure 2 presents the outcomes in homogeneous solutions under physiological conditions. However, under in vivo conditions, excess production of either $^\bullet$NO or $O_2^{\bullet-}$ will serve to trap more of the partner radical, which otherwise would have been consumed by alternative processes instead of accumulating and interfering with the nitration process. For example, SOD lowers substantially the concentration of $O_2^{\bullet-}$, and the rapid transmembrane diffusion and/or cell consumption of $^\bullet$NO operate as a drain of excess $^\bullet$NO.[27] It is also worth noting that thiols are efficient biological scavengers of $^\bullet NO_2$[188] and carbonate ion radicals,[184] and therefore, nitration of tyrosine can be prevented by the presence of relatively low concentrations of thiols,[189] provided the latter have access to the reactive site.

Peroxynitrate is less reactive than peroxynitrite, and under physiological conditions it decomposes into nitrite and O_2 without nitrating or consuming tyrosine.[189] Should $^1O_2$ turn out to be the major product of peroxynitrate decomposition, this might have biological consequences hitherto not considered.

5. Conclusions

The property that fundamentally distinguishes peroxynitrite from peroxynitrate is the strength of the peroxidic O−O bond. This bond is relatively weak in ONOOH or ONOOR, and therefore, most of peroxynitrite chemistry is initiated by or involves O−O bond homolysis. This process produces the highly stabilized $^\bullet NO_2$ radical and a very reactive oxyl radical, namely $^\bullet$OR or $^\bullet$OH. In the presence of CO_2, the oxyl radical produced is $CO_3^{\bullet-}$, known as a very strong oxidant. The biologically toxic effect of peroxynitrite is contingent on the simultaneous presence of these two radical types. While the oxyl radical usually oxidizes a protein, $^\bullet NO_2$ couples with the protein radical to produce a nitrated protein residue, such as 3-nitrotyrosine. Due to the strong O−O bond in peroxynitrate, the latter homolyses along the much weaker N−O bond to yield $^\bullet NO_2$ and a peroxyl radical. Peroxyl radicals are much weaker oxidants and/or hydrogen atom abstractors than oxyl radicals. Consequently, they do not manage to oxidize biological matter, since such a reaction is usually far too slow to compete with the natural decay of peroxynitrate at physiological pH. An interesting possibility is the formation of $^1O_2$ in the latter process, which might have biological consequences. Both peroxynitrite and peroxynitrate produce $^\bullet NO_2$. Were the latter to prove deleterious in its own right, both peroxides could be regarded as toxins. However, in biological systems $^\bullet NO_2$ would appear mainly to add to hemeproteins, such as oxyhemoglobin, producing the oxidized form of the metal ion. Given the ubiquitous presence of reducing enzymes, this process destroys $^\bullet NO_2$ without producing toxins. At the present stage of knowledge, it would then seem that peroxynitrite is a biological toxin, whereas peroxynitrate probably is not.

6. Abbreviations

$ABTS^{2-}$	2,2′-azino-bis(3-ethyl-1,2-dihydrobenzothiazoline 6-sulfonate)
EPR	electron paramagnetic resonance
CIDNP	chemically induced dynamic nuclear polarization

L^1	1,4,8,11-tetraazacyclotetradecane
L^2	*meso*-Me$_6$-[14]ane-N$_4$
Mb	myoglobin
MOONO^{2+}	metal peroxynitrite
NADH	reduced β-nicotinamide adenine dinucleotide
ROO$^•$	alkyl peroxyl radical
RO$^•$	alkoxyl radical
RO$^-$	alkoxide
ROH	alcohol
RONO$_2$	alkyl nitrate
ROONO	alkyl peroxynitrite
ROONO$_2$	alkyl peroxynitrate
TMPyP-Fe(III)	5,10,15,20-tetrakis(*N*-methyl-4′-pyridyl)-porphinatoiron(III)
SOD	superoxide dismutase

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8. References

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