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Peroxynitrite, a Cloaked Oxidant Formed by Nitric Oxide and Superoxide

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Peroxynitrite [oxoperoxonitrate(1-), $ONOO^-$] may be formed in vivo from superoxide and nitric oxide. The anion is stable, but the acid (pK_a = 6.8) decays to nitrate with a rate of 1.3 s^{-1} at 25 °C. The experimental activation parameters of this process are $\Delta H^\ddagger = +18 \pm 1$ kcal/mol, $\Delta S^\ddagger = +3 \pm 2$ cal/(mol·K), and $\Delta G^\ddagger = +17 \pm 1$ kcal/mol. Peroxynitrite (or its protonated form) oxidizes some compounds such as thiols and thioethers in a bimolecular reaction. The reactions with glutathione and cysteine have activation enthalpies of 10.9 and 9.7 kcal/mol, respectively, which are lower than that of the isomerization reaction. Peroxynitrite reacts with other compounds such as dimethyl sulfoxide and deoxyribose in a unimolecular reaction for which the activation of peroxynitrite is rate-limiting. In theory, activation could involve (1) heterolysis to OH^- and NO_2^+ ($\Delta_{rxn}G^{\circ\prime}$ = 13 kcal/mol at pH 7) or (2) homolysis to $^\bullet OH$ and $^\bullet NO_2$ ($\Delta_{rxn}G^\circ$ = 21 kcal/mol), and these processes also could be involved in the isomerization to nitrate. However, thermodynamic and kinetic considerations indicate that neither process is feasible, although binding to metal ions may reduce the large activation energy associated with heterolysis. An intermediate closely related to the transition state for isomerization of ONOOH to $HONO_2$ may be the strongly oxidizing intermediate responsible for hydroxyl radical-like oxidations mediated by ONOOH. Thus, peroxynitrite reacts with different compounds by at least two distinct mechanisms, and the hydroxyl radical is not involved in either.

Introduction

Superoxide reacts rapidly with nitric oxide to form the peroxynitrite anion (*1*) with a rate constant of (3.7 ± 1.1) × 10^7 M^{-1} s^{-1} (*2*). This reaction probably occurs in vivo (*3*). Nitric oxide, which is formed from the enzymic (*4–10*) conversion of L-arginine to L-citrulline via an N^ω-hydroxy-L-arginine intermediate (*11*), plays a critical role in cell regulation and communication (*12*). Nitric oxide is involved in relaxation of vascular smooth muscle (*13*), platelet aggregation (*14*), inhibition of protein synthesis (*15*), neurotransmission (*16*), mediation of glutamate neurotoxicity (*8*), and lysis of tumor cells (*17*). Excess production of nitric oxide can be toxic, as follows from studies of septic shock (see ref 18 for review).

Superoxide is formed by a variety of organelles and cells (see ref 19 for review). Stimulation of neutrophils and macrophages increases the rate of production of nitric oxide and superoxide (*12*, *20–22*), and the formation of peroxynitrite appears to proceed quantitatively in activated rat alveolar macrophages (*23*).

Peroxynitrite carries out reactions that can harm cells: it initiates lipid peroxidation (*24*) and reacts with sulfhydryls (*25*) and methionine (*26*), and it may play a role in reperfusion injury, where ischemic endothelium produces superoxide and nitric oxide when oxygen is readmitted (*27*). At low pH, peroxynitrite is protonated (pK_a = 6.8, see below), and in this form it hydroxylates and nitrates aromatic compounds (*28*, *29*). In addition, peroxynitrite is relatively long-lived, which allows it to reach critical targets. There is no consensus on the mechanism by which peroxynitrite or peroxynitrous acid carries out its deleterious reactions. In the past three mechanisms have been proposed: (i) peroxynitrous acid decomposition to the hydroxyl radical (*30*, *31*), (ii) decomposition to the nitronium ion (NO_2^+) (*32*), and (iii) a direct attack by peroxynitrous acid (*32*). Currently, it is generally assumed that peroxynitrite yields the hydroxyl radical (*3*, *33*, *34*). The instability of peroxynitrous acid may also have contributed to the fact that different values for its dissociation constant and its rate of decomposition have been reported (*3*, *35*, *36*).

Few thermodynamic data are available on which calculations of peroxynitrite reactions can be based. We calculate Gibbs energies of formation for a variety of species and evaluate activation energies in order to discuss possible reaction mechanisms. Our kinetic and thermodynamic data show that peroxynitrous acid is unlikely to dissociate into the hydroxyl radical and nitrogen dioxide, but is a strongly oxidizing compound by itself.

Experimental Procedures

Chemicals. Oxoperoxonitrate(1-)[1] was synthesized in a quenched-flow reactor from sodium nitrite and hydrogen peroxide

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[1] In the literature, $ONOO^-$ is named peroxynitrite or peroxonitrite. Here recommended (*37*) names will be used, as follows: oxoperoxonitrate(1-) for peroxynitrite (O=NOO$^-$), hydrogen oxoperoxonitrate for peroxynitrous acid (O=NOOH), nitrogen monoxide for nitric oxide ($^\bullet$NO), nitryl cation for the nitronium cation (NO_2^+), and hydron instead of proton to indicate the naturally occurring mixture of hydrogen isotopes. The word proton is now reserved for the isotopically pure $^1H^+$ ion.

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as previously described (*3*, *38*). Oxoperoxonitrate(1−) is an oxidizing agent and should be treated with caution. Treatment of oxoperoxonitrate(1−) with manganese dioxide to remove residual hydrogen peroxide was generally avoided to reduce metal contamination. Results obtained both with and without manganese dioxide treatment were generally comparable when all buffers contained 0.10 mM dtpa.[2] The concentration of oxoperoxonitrate(1−) was determined at 302 nm in 1 M sodium hydroxide [ϵ = 1670 ± 50 M^{-1} cm^{-1} (*32*)]. To minimize trace metal contamination, phosphate buffers were passed through a mixed resin bed consisting of Dowex anion resin, Chelex 100 resin, and then charcoal to remove organics.

Instrumentation. Kinetic measurements were made with two stopped-flow spectrophotometers: a Hi-Tech instrument (Birmingham, U.K.) with a mixing time of less than 2 ms, and an instrument manufactured by Kinetic Instruments, Inc. (Ann Arbor, MI), and On-Line Instrument Services (Jefferson, GA) with a mixing time of less than 1.5 ms. Buffers and solutions were rigorously degassed with helium. Stock oxoperoxonitrate(1−) was diluted to 1 mM with deionized water containing 0.10 mM dtpa immediately before transfer to the stopped-flow spectrophotometer. The temperature was maintained to within 0.1 °C. The pH was measured at the outlet to detect changes caused by the addition of alkaline oxoperoxonitrate(1−). The rate of oxoperoxonitrate(1−) disappearance was followed at 302 nm. The activation energy for the decomposition of oxoperoxonitrate was determined at pH 5.0 ± 0.1 (0.50 M acetate buffer, 5.0 mM dtpa) from an Arrhenius plot over a temperature range of 2.0–62.5 °C. Temperature dependences were determined at other pH's and in the presence of 0.10 M *tert*-butyl alcohol. The activation enthalpy and entropy were calculated as described in ref 39.

Results

Energetics. In order to estimate the thermodynamics of reactions of oxoperoxonitrate(1−), it is necessary to calculate the Gibbs energies of formation of oxoperoxonitrate(1−) and its products. The thermodynamic values derived below pertain to pH 7 and a 1 M concentration reference state, even in the case of gases. For that reason these energies and reduction potentials do not represent standard values, as indicated by the prime in $\Delta_f G°'$, and $E°'$. The thermodynamic (*40*) and kinetic (*41*) properties of superoxide have been described elsewhere. Nitrogen monoxide is a mildly oxidizing radical when the product, NO^-, is in the triplet state [$E°'(^•NO/^3NO^-)$ = 0.39 V (*42*)], as shown by its ability to oxidase thiols to disulfides (*43*) and Cu(I) in superoxide dismutase (*44*). In the presence of excess oxygen, nitrogen monoxide reacts to form initially the nitrosyldioxyl radical, $ONOO^•$ (*45*), which reacts with another nitrogen monoxide to form nitrogen dioxide. Nitrogen monoxide binds to Fe(II) in heme proteins (*46*), which in the case of cytosolic guanylate cyclase leads to activation (*47*), to release of cyclic guanosine monophosphate, and ultimately to relaxation of arteries and veins.

In the following sections a number of thermodynamic quantities are estimated. These are collected in Table I.

$ONOO^-$ and ONOOH. The standard Gibbs energy of formation of the oxoperoxonitrate(1−) anion is not known, but its enthalpy of formation is given as −10.8 ± 2.0 kcal/mol at 274 K (*48*). The National Bureau of Standards tables list a value of −10.7 kcal/mol 298 K (*49*). Its entropy ($S°$) is likely to be larger than that of nitrate, 35.0 cal/(mol·K), mainly because oxoperoxonitrate(1−) is not symmetric. Other possible corrections are probably small: the molar mass of oxoperoxonitrate(1−) and the number of bonds are the same as for nitrate; as to vibrations, the change from a N–O vibration in nitrate to an O–O vibration in oxoperoxonitrate(1−) will have a small effect on the entropy. According to Benson (*39*) the difference between the absolute entropies of $NO_3^•$ and $ONOO^•$ is 9.6 ± 1.0 cal/(K·mol). It is assumed here that the same difference applies to the anions. These considerations lead to an entropy ($S°$) of oxoperoxonitrate(1−) at 298 K of 45 cal/(K·mol). Together with the absolute entropies of dinitrogen, dioxygen, and dihydrogen, 45.8, 49.0, and 31.2 cal/(K·mol), respectively (*49*), one calculates a standard entropy of formation of −68 cal/(K·mol) for oxoperoxonitrate(1−). This value and the standard enthalpy of formation of oxoperoxonitrate(1−) of −10.7 kcal/mol (*49*) yield a standard Gibbs energy of formation of 10 kcal/mol for the oxoperoxonitrate(1−) anion. With random errors of 2 kcal/mol in $\Delta_f H°$ (*48*) and an estimated 1 kcal/mol from the $T\Delta_f S°$ term the uncertainty in the Gibbs energy is expected to be 2 kcal/mol. The hydronated form, ONOOH, has a pK_a of 6.8 as reported below and in refs 2, 25, and 36. Thus, the standard Gibbs energy of formation of ONOOH is 1 ± 2 kcal/mol.

From the standard Gibbs energy of formation of $ONOO^-$ derived here and those of $^•NO_2$(aq) and H_2O, 15.05 kcal/mol (*42*) and −56.7 kcal/mol (*49*), respectively, one calculates that the reduction potential corresponding to eq 1a is 1.4 ± 0.1 V at pH 7 via $\Delta G°' = -nFE°'$. Similarly,

$$ONOO^- + 2H^+ + e^- \rightarrow {}^•NO_2(aq) + H_2O \qquad (1a)$$

one calculates a value of 1.20 ± 0.05 V at pH 7 for the two-electron half-reaction by combining the reduction potential of eq 1a with the reduction potential of the $^•NO_2(aq)/NO_2^-$ couple, 0.99 V (see Figure 1):

$$ONOO^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O \qquad (1b)$$

Alternatively, if one-electron reduction results in hydrogen peroxide and nitrogen monoxide, equation 2, the reduction

$$ONOO^- + 2H^+ + e^- \rightarrow {}^•NO(aq) + H_2O_2 \qquad (2)$$

Table I. Thermodynamic Quantities Related to Oxoperoxonitrate(1−)

formation of $ONOO^-$			
$\Delta_f H°$ (kcal/mol)[a]	$\Delta_f G°$ (kcal/mol)		$S°$ [cal/(mol·K)]
−10.7 ± 2.0	+10 ± 2		+45
ionization of ONOOH			
$\Delta H°$ (kcal/mol)	$\Delta G°$ (kcal/mol)		$\Delta S°$ [cal/(mol·K)]
0	+9.2		−31
standard Gibbs energies of formation (kcal/mol)			
ONOOH(aq)	+1 ± 2	OH^+(aq)	+106 ± 10
ONOOH(g)	+9 ± 2	$ONOO^•$(aq)	+20 ● 2
NO_2^+(aq)	+52 ± 4	$^•NO_3$(aq)	+31 ± 2
bond dissociation (kcal/mol)			
$\Delta H°$(ONOOH)(aq)	+17 ± 1	$\Delta H°$(ONOOH)(g)	+23 ± 3
reduction potentials (V)			
$E°'[ONOO^-, 2H^+/^•NO_2(aq)]$ (pH 7)			+1.4 ± 0.1
$E°'[ONOO^-, 2H^+/^•NO(aq), H_2O_2]$ (pH 7)			0 ± 0.1
$E°'[ONOO^-, 2H^+/^•NO_2^-]$ (pH 7)			+1.20 ± 0.05
$E°'[NO_2^+/^•NO_2(aq)]$			+1.6 ± 0.2
$E°'[ONOO^•(aq)/ONOO^-]$			+0.43 ± 0.13

[a] See ref 46.

[2] Abbreviation: dtpa, *N,N,N'N'',N''*-diethylenetriaminepentaacetate; *e*, unit charge, 1.6 × 10^{-19} C.

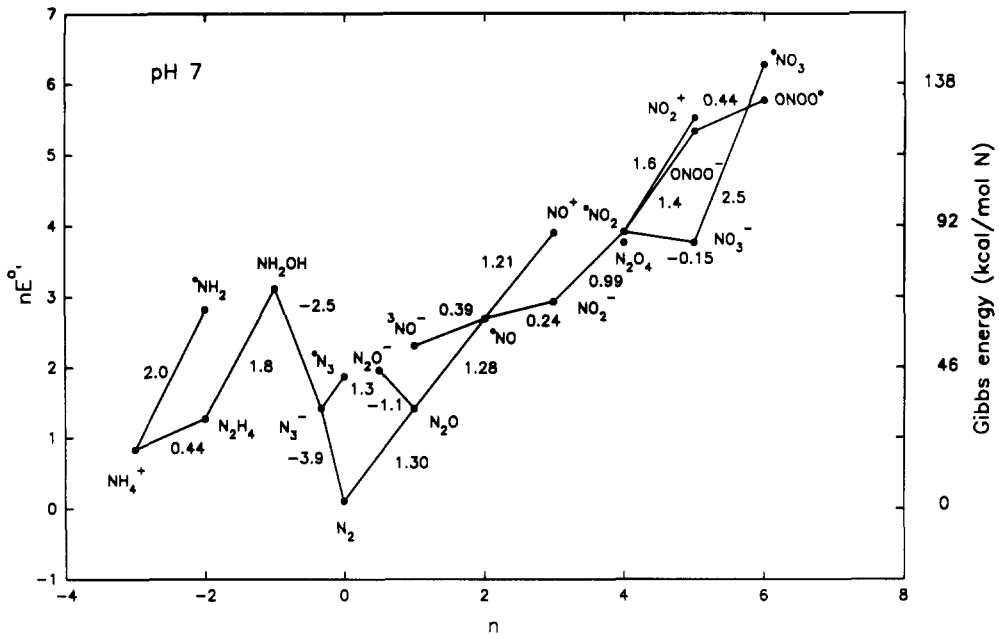


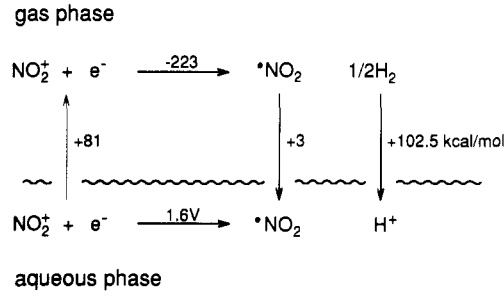
Figure 1. Oxidation-state diagram of nitrogen at pH 7, modified after ref 73. Abscissa: oxidation state, n, of the element shown. Ordinate, left: n times the reduction potential in volts, relative to the normal hydrogen electrode; ordinate, right: $\Delta_{rxn}G^{\circ\prime}$ per mole of nitrogen to form the compound of interest from nitrogen, water, and hydrogen at pH 7. The numbers refer to the slopes of the lines and represent reduction potentials. The assignment of an oxidation state of +6 to nitrogen in ONOO• follows from the standard −2 oxidation state assigned to oxygen. Conditions: pH 7, 298 K, and 1 molal concentrations for all compounds, including gases. Data for this diagram were obtained from refs 42, 49, and 74, and as discussed in the text.

potential is 0.0 V at pH 7. Equation 1a is more favourable, and one-electron transfer to a peroxide results usually in scission of the O–O bond.

ONOO•. The standard Gibbs energy of formation of the nitrosyldioxyl radical, ONOO•(g), the adduct of nitrogen monoxide and dioxygen, is 25.5 kcal/mol, on the basis of its standard enthalpy of formation of 17.6 kcal/mol and its absolute entropy of 70 cal/(mol·K), respectively (*39*). We estimate the Gibbs energy of hydration at −6 kcal/mol, on the basis of the number of possible hydrogen bonds (*50*). Thus, the Gibbs energy of formation of ONOO•(aq) is 20 ± 2 kcal/mol, and E°(ONOO•/ONOO⁻) is 0.43 ± 0.13 V. Experimental support for this value exists. Alkaline permanganate oxidizes oxoperoxonitrate and forms manganate (*51*) and—we assume—the nitrosyldioxyl radical, ONOO•. When this titration is followed potentiometrically, a potential of 0.20 V vs the calomel electrode is measured at the beginning of the experiment (*52*), in quite good agreement with the 0.43 V vs the normal hydrogen electrode estimated above. Since this potential represents the difference between the Gibbs energies of formation of ONOO• and ONOO⁻, it seems likely that these estimated thermodynamic quantities are approximately correct.

NO_2^+. The standard Gibbs energy of formation of the nitryl cation and the standard reduction potential of the NO_2^+(aq)/•NO_2(aq) couple are estimated from the cycle shown in Scheme I. The Gibbs hydration energy of NO_2^+ is estimated to be −81 kcal/mol, close to that of the potassium cation (*53*). The energy of ionization is the difference in the enthalpies of formation (*49*) of nitrogen dioxide and the nitryl cation. It should be safe to neglect the entropies. The solvation energy of nitrogen dioxide is calculated from refs 49 and 42, and the Gibbs energy of formation of H^+ is from ref 54. The cycle depicted in

Scheme I



Scheme I gives the desired half-reaction:

$$NO_2^+(aq) + e^- \rightarrow {}^{\bullet}NO_2(aq) \qquad +1.6\ V \qquad (3)$$

The error in this reduction potential is estimated to be 0.2 V. A slightly lower estimate of 1.51 V for $E^{\circ\prime}$(NO_2^+/•NO_2), based on the concentration of the nitryl cation in a mixture of nitric acid and concentrated sulfuric acid, has been given by Stanbury (*42*). The energetics of eq 3 show that the Gibbs energy of formation of NO_2^+(aq) is 37 kcal/mol higher than that of •NO_2(aq), which is 15 kcal/mol (*42*). Thus, $\Delta_f G^{\circ}[NO_2^+(aq)]$ is 52 kcal/mol.

The thermodynamic values derived above make it possible to construct an oxidation-state diagram of nitrogen that allows a visual comparison of the reduction potentials of the NO_2^+/•NO_2 and the ONOO⁻/•NO_2 couples (Figure 1) with those of other nitrogen compounds. The slopes in this diagram are proportional to reduction potentials. Of all the compounds that are physiologically relevant, NO_2^+ and ONOO⁻ are the strongest oxidizing species.

OH^+. The Gibbs energy of OH^+(aq) is estimated in manner similar to that of the nitryl cation. The Gibbs solvation energy of •OH is −2 kcal/mol (*50*), the ionization energy is 308 kcal/mol (*49*), and the Gibbs solvation energy of OH^+ is estimated as follows. The internuclear distance in OH^+ is 1.03 Å (*55*). Taking that as the radius, a hydration Gibbs energy of 108 kcal/mol follows from

Table II. Gibbs Energy Changes for Reactions Involving Oxoperoxonitrate(1−)[a]

reaction	$\Delta_{rxn}G^{\circ\prime}$ (kcal/mol)[b]
$^{\bullet}NO + O_2^{\bullet-} \rightarrow ONOO^-$	−22
$^3NO^- + O_2 \rightarrow ONOO^-$	−9[c]
$HNO_2 + H_2O_2 \rightarrow ONOOH + H_2O$	−10[d]
$ONOOH \rightarrow H^+ + NO_3^-$	−36
$ONOOH \rightarrow {}^{\bullet}OH + {}^{\bullet}NO_2$	+21[e]
$ONOOH \rightarrow OH^- + NO_2^+$	+13
$ONOOH + H^+ \rightarrow H_2O + NO_2^+$	+4[f]
$ONOOH \rightarrow NO^+ + HOO^-$	+35
$ONOOH \rightarrow OH^+ + NO_2^-$	+97
$2ONOOH \rightarrow H_2O + ONOO^{\bullet} + {}^{\bullet}NO_2$	−23[g]
$2ONOOH \rightarrow H_2O_2 + ONOO^{\bullet} + {}^{\bullet}NO$	+10
$2ONOO^- \rightarrow O_2 + 2NO_2^-$	0[g]
$ONOO^- + {}^{\bullet}NO \rightarrow {}^{\bullet}NO_2 + NO_2^-$	−27
$ONOOH + O_2^{\bullet-} + H^+ \rightarrow {}^{\bullet}NO_2 + O_2 + H_2O$	−36
$ONOOH + H_2O_2 \rightarrow {}^{\bullet}NO_2 + O_2^{\bullet-} + H_2O + H^+$	−11

[a] There energies pertain to neutral pH and to all species dissolved in water. [b] The uncertainty is 2 kcal/mol, as discussed in the text. [c] Experimental evidence has been obtained for this reaction (72). [d] Formation reaction, at pH 0. [e] Due to the pK_a of 6.8 this reaction has, within the uncertainty, the same $\Delta r_{xn}G^{\circ}$ at pH 0. [f] At pH 0 $\Delta_{rxn}G^{\circ}$ is −5 kcal/mol. [g] Although favorable, the first-order decay of hydrogen oxoperoxonitrate indicates that this reaction does not occur significantly at low temperatures. However, upon heating, oxygen evolution has been observed (*51*).

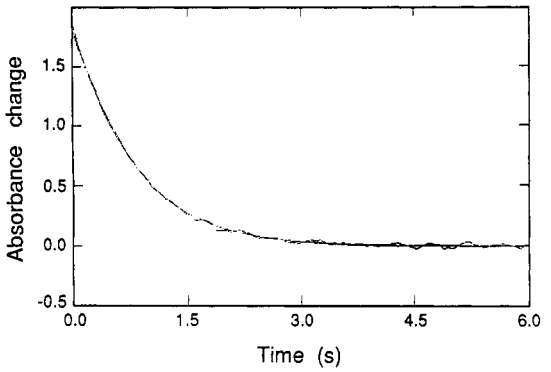


Figure 2. Decomposition of oxoperoxonitrate(1−) follows first-order kinetics. Conditions: wavelength, 302 nm; pH 5.0 ± 0.1; temperature, 25 °C.

interpolation between the Gibbs hydration energies of the sodium and lithium cations (*53*). These estimates result in a standard Gibbs energy of formation of OH^+(aq) of approximately 106 kcal/mol and a reduction potential, $E^{\circ\prime}(OH^+/{}^{\bullet}OH)$, of 4.3 V. Due to the uncertainties in the radius and the solvation energy, the error in these values is estimated at 10 kcal/mol and 0.4 V, respectively.

Decay of ONOOH. In principle, hydrogen oxoperoxonitrate can (i) isomerize to nitric acid, (ii) undergo homolysis or heterolysis of the peroxide bond, or (iii) dismutase. These reactions and their Gibbs energy changes are shown in Table II. Only the isomerization and the disproportionation reaction to $^{\bullet}NO_2$, $ONOO^{\bullet}$, and H_2O, or to O_2 and NO_2^-, have negative Gibbs energies (see also Figure 1). Due to the high Gibbs energy of formation of the hydroxylium ion, heterolysis to form OH^+ and NO_2^- is unfavorable by 97 kcal/mol (Table II). The Gibbs energy of the transition state will be approximately 30 kcal/mol higher, as shown above for hydroxide and nitryl, and so we will not consider this pathway further.

Kinetics. The decomposition of oxoperoxonitrate(1−) measured at 302 nm by the stopped-flow technique follows first-order kinetics (Figure 2) over the limits of the pH range tested (*56*). The rate of decomposition is not affected

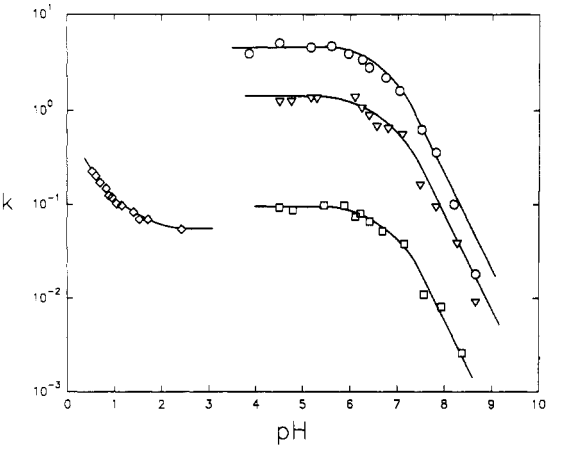


Figure 3. Rate of decomposition of oxoperoxonitrate(1−) as a function of pH at 5 °C (□), 25 °C (▽), and 37 °C (○). The data below pH 3, obtained at 1.5 °C, are from ref 35.

by addition of 100 mM DMSO or by mannitol (not shown). The first-order rate constant as a function of pH is described by eq 4 (*56*), where k is the observed first-order

$$k = \frac{k_{HA}[H^+]}{[H^+] + K_a} \tag{4}$$

rate constant of oxoperoxonitrate(1−) disappearance at a given pH, k_{HA} is the first-order rate constant for hydrogen oxoperoxonitrate decomposition, and K_a is its acid dissociation constant. First-order rate constants for oxoperoxonitrate(1−) decomposition measured over a pH range from 4 to 8.5 indicate that the pK_a is 6.80 at 5 and 25 °C and 6.75 at 37 °C, as shown in Figure 3. The rate constants, k_{HA}, for the decay of hydrogen oxoperoxonitrate are 0.095 s^{-1}, 1.3 s^{-1}, and 4.5 s^{-1}, at 5, 25, and 37 °C, respectively, which correspond to a half-lives of 7.3, 0.53, and 0.15 s. Oxoperoxonitrate(1−) is contaminated with nitrate, nitrite, and hydrogen peroxide, but addition of any of these components up to a concentration of 50 mM has no effect on the rate of oxoperoxonitrate(1−) decomposition as previously reported (*32*).

The activation energy for hydrogen oxoperoxonitrate decomposition is calculated from the temperature dependence (2.0–62.5 °C) to be 18.5 kcal/mol at pH 5.0 ± 0.1 (Figure 4). The enthalpy and entropy of activation are 17.8 kcal/mol and 2.0 cal/(mol·K), respectively. In the presence of 0.1 M *tert*-butyl alcohol and over a smaller temperature range (5–45 °C) activation parameters are found that are not significantly different: $\Delta H^{\ddagger}$ is 18.4 kcal/mol and $\Delta S^{\ddagger}$ is 3.8 cal/(mol·K). From these measurements we arrive at best values of 18 ± 1 kcal/mol for the activation enthalpy, 3 ± 2 cal/(mol·K) for the activation entropy, and 17 ± 1 kcal/mol for the Gibbs activation energy.

In contrast, cysteine reacts in a bimolecular fashion with oxoperoxonitrate(1−) with a rate constant of 5.9×10^3 M^{-1} s^{-1} at 37 °C and pH 7.4 (*25*). We find that glutathione reacts similarly. The activation enthalpies calculated for the oxidation of cysteine and glutathione are 10.9 and 9.7 kcal/mol, respectively (Figure 5).

Discussion

Decomposition. The pK_a of hydrogen oxoperoxonitrate is 6.8 at 25 °C, virtually identical to the values obtained at 5 °C, 37 °C (Figure 3), and 2 °C (*35*). We

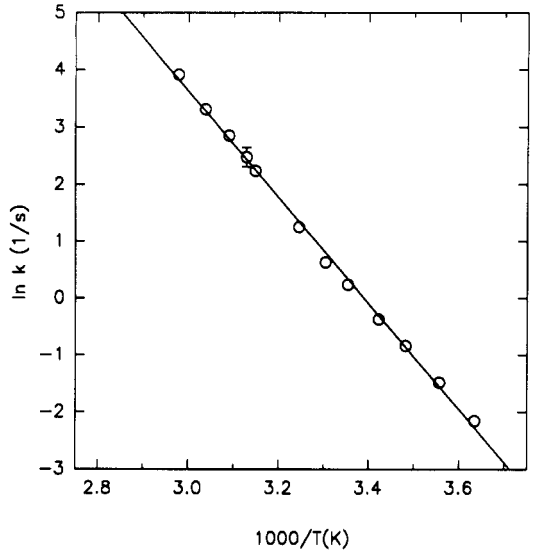


Figure 4. Arrhenius plot of the first-order decomposition of hydrogen oxoperoxonitrate at pH 5.0 ± 0.1. The temperature was varied from 2.0 to 62.5 °C.

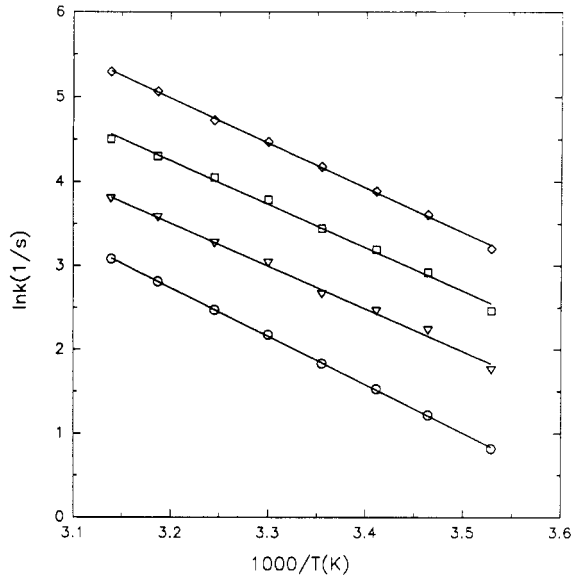


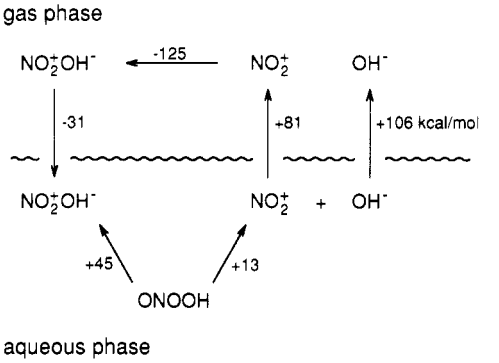
Figure 5. Arrhenius plot of the bimolecular reaction of oxoperoxonitrate(1−) with glutathione and cysteine at pH 7.5. The concentration of oxoperoxonitrate(1−) was 1.0 mM, the glutathione concentration was 10 mM (○), and the cysteine concentrations were 5 (▽), 10 (□), and 20 mM (◇).

therefore conclude that the standard enthalpy of dissociation is close to 0 kcal/mol.

Our value for the activation enthalpy for the decomposition of hydrogen oxoperoxonitrate, 18 ± 1 kcal/mol, is consistent with the 19.6 kcal/mol determined by Benton and Moore (*35*) in dilute perchloric acid. Their value for the entropy of activation, 7.4 cal/(mol·K), is higher than that reported here, 3 cal/(mol·K), possibly because these authors obtained kinetic data at only two temperatures, 1.5 and 38.5 °C. In contrast to the activation enthalpy of 18 kcal/mol given above, Hughes and Nicklin (*32*) measured a value of 12 kcal/mol when the decay of oxoperoxonitrate(1−) was observed near pH 12. This lower activation energy may have been due to trace metal catalysis.

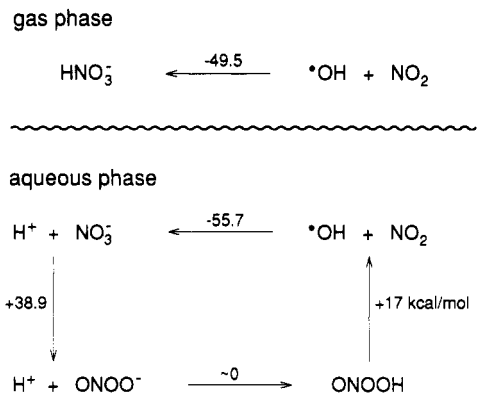
The only modes of decay of hydrogen oxoperoxonitrate that are thermodynamically favorable are the isomerization to nitrate and a dismutation, either to nitrogen dioxide and the nitrosyldioxyl radical or to nitrite and oxygen (Table II). Oxygen evolution from oxoperoxonitrate(1−) or its hydronated form has been observed to a small extent upon heating (*51*) and at room temperature during lipid peroxidation studies (*24*). The kinetics of the decay of hydrogen oxoperoxonitrate do not show evidence for dismutation: the disappearance of hydrogen oxoperoxonitrate is strictly a first-order reaction as shown by Figure 2. The isomerization reaction might possibly proceed through heterolysis or homolysis, and these pathways will be evaluated below.

Scheme II



ONOOH → OH^- + NO_2^+. The hydroxide anion and nitryl cation are attractive as intermediates, because their formation from hydrogen oxoperoxonitrate is energetically feasible, $\Delta_{rxn}G^\circ$ = +13 kcal/mol. However, the initial charge separation leads to ONO^+OH^-, which is unfavorable in water, since solvation of the anion and cation by dipolar water molecules is considerably more favorable than the sum of the electrostatic energy gained by bringing NO_2^+ and OH^- together and solvation of the dipole so formed (see Scheme II). For the calculation of the electrostatic interaction energy the radii of hydroxide and nitryl were used, and the result is identical within the error to that obtained by Nangia and Benson (*57*). The Gibbs solvation energy of the ONO^+OH^- dipole is calculated with an equation derived by Kirkwood (*58*). This approach underestimates solvation energies, and an arbitrary 15%, as recommended in (*57*), has been added to yield a Gibbs solvation energy at −31 kcal/mol. The result is that the initial charge separation is unfavorable by 45 kcal/mol. Due to the underestimation of the dipolar solvation energy this activation energy may be overestimated. Nevertheless, formation of the ion pair seems unlikely. The dipolar solvation pitfall can be avoided if hydrogen oxoperoxonitrate were to be hydronated and decay to water and the nitryl cation, which might occur below pH 2. Benton and Moore (*35*) reported that in addition to the hydron-independent pathway (from pH 2.5 to pH 5.5) hydrogen oxoperoxonitrate decomposes by an H^+-dependent mechanism below pH 2 (see Figure 3). We propose here that the low-pH decomposition involves the formation of NO_2^+, the nitryl cation, which upon rapid hydration forms nitrate. The observation of an accelerated decay at low pH allows us to make a different estimate of the activation energy of the heterolysis reaction. The Gibbs activation energy, calculated from the activation enthalpy and entropy at 14.8 kcal/mol and 5.3 cal/(mol·K) obtained at low pH (*35*), is 13 kcal/mol. Together with the work of changing the pH from 7 to 0, 9.5 kcal/mol, we arrive at a total Gibbs activation energy of 23 kcal/mol. This value is higher than that calculated from our experiments, 17 kcal/mol. We

Scheme III



therefore conclude that heterolysis is not a likely step in the decomposition of hydrogen oxoperoxonitrate at neutral pH.

ONOOH → •OH + •NO₂. Since homolysis results in two radicals, the activation parameters of this process should equal those of the bond dissociation reaction. An estimate for the enthalpy of this process is obtained from a cycle proposed by Mahoney (*31*) (see Scheme III). He estimated the enthalpy of O–O bond scission to be approximately 10 kcal/mol by assuming that the standard enthalpy of the reaction of the hydroxyl radical with nitrogen dioxide to form nitric acid is the same in the gas phase as in the aqueous phase. The bond dissociation energy in water could be calculated if the enthalpy of formation of nitrogen dioxide in water were known. This quantity can be estimated as follows. The standard enthalpy of formation of nitrogen dioxide in the gas phase is 7.9 kcal/mol (*49*). With an estimated enthalpy of solvation of −5 kcal/mol, similar to those of CO_2, ClO_2, SO_2, and O_3 (*49*), we arrive at the value of 3 kcal/mol for the enthalpy of formation of aqueous nitrogen dioxide. The enthalpy of ionization of hydrogen oxoperoxonitrate is close to 0 kcal/mol. With these values the enthalpy of homolysis of hydrogen oxoperoxonitrate in water is 17 kcal/mol.

We now estimate the enthalpy of the bond dissociation reaction in the gas phase. Standard Gibbs energies are known for the hydroxyl radical and nitrogen dioxide in the gas phase, 8.2 and 12.3 kcal/mol, respectively (*49*). The Gibbs energy of formation of ONOOH(aq) is 1 ± 2 kcal/mol. If we assume that this species has the same Gibbs energy of hydration as hydrogen peroxide, −7.8 kcal/mol (*40*), then $\Delta_f G°(ONOOH)_g$ is 9 kcal/mol and the Gibbs energy change of homolysis in the gas phase is +11 kcal/mol. The $T\Delta S°$ term amounts to +9 to +10 kcal/mol (*39*, *59*), which leads to an estimated 21 ± 3 kcal/mol for the bond dissociation enthalpy in the gas phase, in good agreement with the 20 kcal/mol estimated from a comparison of the bond energies a number of peroxides (*60*), and the 16 kcal/mol which followed from an ab initio calculation (*61*). This bond dissociation enthalpy appears to be one of the smallest values known for a peroxide bond, including those of peresters (*62*). The calculated Gibbs activation energies for homolysis and heterolysis, and that of the experimentally determined isomerization, are shown in Figure 6.

Does the Decomposition Reaction of Hydrogen Oxoperoxonitrate Proceed via a Free Hydroxyl Radical? It has been argued that the reactions of hydrogen oxoperoxonitrate, including its conversion to nitrate,

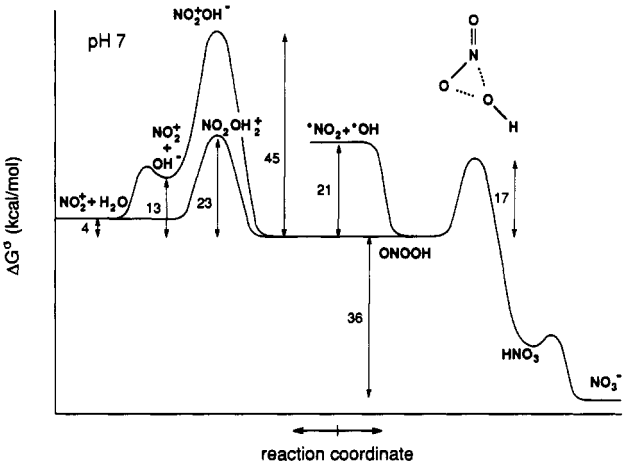


Figure 6. Calculated Gibbs energies of homolysis and heterolysis of ONOOH in comparison to the experimentally determined Gibbs activation energy of the isomerization reaction.

proceed via the hydroxyl radical (*3*, *29*–*31*). The activation enthalpy calculated above for decomposition of hydrogen oxoperoxonitrate to the hydroxyl radical and nitrogen dioxide in water is 17 kcal/mol. Over the pH range of 4–8 we experimentally determined the activation enthalpy for isomerization of oxoperoxonitrate to be 18 ± 1 kcal/mol (Figure 4), which would tend to support the notion that homolysis occurs and hydroxyl radicals are formed. We will now present two arguments that this is not the case.

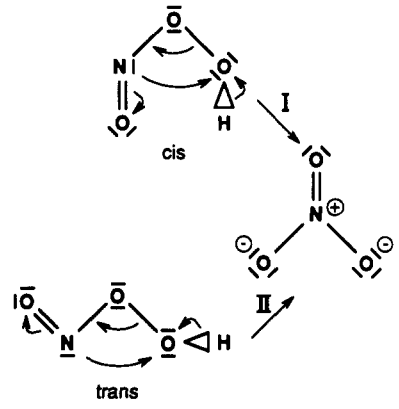
Let us assume that the decomposition of hydrogen oxoperoxonitrate involves homolysis, followed by the rapid reaction of the hydroxyl radical with nitrogen dioxide to form nitrate. The forward rate constant for oxoperoxonitrate(1−) decomposition is 1.3 s^{-1} at 25 °C, and it is further assumed that this is the rate constant for the rate-limiting homolysis. The rate constant for the reverse process, namely, the reaction of the hydroxyl radical with nitrogen dioxide to form hydrogen oxoperoxonitrate, can then be calculated from

$$\Delta_{rxn}G° = -RT \ln (k_f/k_r) \qquad (5)$$

to be in the range of 10^{13}–10^{15} M^{-1} s^{-1}. Such a rate constant would not only exceed the experimentally determined rate constant of 1.3 × 10^9 M^{-1} s^{-1} (*36*), but also the diffusion-limited reaction rate constant of 10^{10}–10^{11} M^{-1} s^{-1}. It is therefore most unlikely that hydrogen oxoperoxonitrate decomposes to form free hydroxyl radicals.

Furthermore, the entropy of activation is small, 3 ± 2 cal/mol·K). Activation entropies for homolysis of RO–OR bonds are generally larger, 12 cal/(mol·K) (*39*). Some peroxide decompositions proceed through an induced decomposition. In such a case the experimentally determined activation entropy and enthalpy are both too small, because these parameters are those of the induced chain reactions (*63*). In the present case of hydrogen oxoperoxonitrate, hydroxyl radical scavengers like DMSO and *tert*-butyl alcohol do not affect the rate of decomposition; consequently, no *induced* decomposition occurs, and the values for the activation enthalpy and entropy should be correct as measured. The small positive activation entropy observed here must reflect rigidity in the transition state (*39*). A number of transition states would appear to satisfy these requirements, and in light of the arguments against a free hydroxyl radical and nitryl cation, we propose that an intermediate with a structure close to that of the transition state is the oxidizing species. The first mech-

Scheme IV



anism involves a cyclic conversion of hydrogen oxoperoxonitrate in the cis configuration[3] (*64*) without isolatable intermediates (reaction I in Scheme IV). The second mechanism is similar to the first, with the molecule in the trans configuration (reaction II).

A potential transition state fitting the requirements of a low entropy of activation and an activation enthalpy for the isomerization of *trans*-oxoperoxonitrate was identified with the help of the computer program MOPAC and the PM3 parameter set (*65*). The transition state is reached when the O–O–N angle is reduced from 111° to about 80° and the O–O bond is stretched from 1.4 to about 1.8 Å. These distortions are thought to occur naturally. Calculated frequencies of the normal mode O–O–N bending vibration, which is coupled with O—N=O torsion, and the O–O stretching vibration are approximately 360 and 950 cm^{-1}, respectively, at the RMP2/6-31G* level (*61*). Of these the O–O stretching vibration has been experimentally observed (*66*). The state with the reduced O–O–N angle and the lengthened O–O bond is 18.3 kcal/mol higher in enthalpy than the trans configuration which lies 44 kcal/mol above nitric acid. These values refer to enthalpies in the gas phase. The partial charges are –0.135*e* and –0.190*e* on the terminal and middle oxygens, respectively. These charges indicate that, indeed, no significant separation of charge into OH^- and NO_2^+ occurs in the putative transition state (Figure 7). Ab initio calculations indicate that the cis configuration is lower in energy than the trans configuration by 1.3 kcal/mol (*61*). The agreement between the experimentally determined and the calculated activation enthalpy is remarkable. In contrast, construction of a transition state from the cis configuration fails: the oxygen atoms next to the nitrogen prevent the terminal oxygen from approaching the nitrogen atom.

A transition state, lying approximately 17 kcal/mol above that of undistorted hydrogen oxoperoxonitrate, will be correspondingly more oxidizing. Thus, if $E^{\circ\prime}$(ONOOH/ $^{\bullet}NO_2$,H_2O) is 1.4 ± 0.1 V at pH 7, then $E^{\circ\prime}$($ONOOH_{int}$/ $^{\bullet}NO_2$,H_2O) is 0.7 V higher, or 2.1 V. For comparison, the best value for $E^{\circ\prime}$($^{\bullet}OH/H_2O$) at pH 7 is 2.31 V (*40*). A target molecule in the vicinity of hydrogen oxoperoxonitrate in the activated state will be oxidized as though a free hydroxyl radical were present. This transoid activated hydrogen oxoperoxonitrate may account for the observation that only a fraction of hydrogen oxoperoxonitrate yields

[3] For the assignment of cis and trans in O=NOO′H we define the reference plane as that containing the N–O bond, perpendicular to the O=NOO′ plane. In *cis*-O=NOO′H the O=N and the O′–O bonds are on the same side of this plane, whereas in the trans configuration they are on opposite sides.

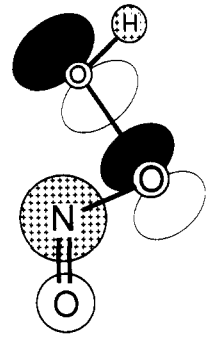


Figure 7. Putative oxidizing intermediate generated with the program MOPAC. The structure shown is likely to be close to that of the transition state of the isomerization reaction. Shown are the empty σ-antibonding orbitals of the peroxide oxygens. The size of these oxygens is proportional to their charges, which are –0.135*e* and –0.190*e* on the terminal and middle oxygens, respectively.

hydroxyl radical-like products under optimal trapping conditions (*3*, *33*); the remainder decomposes directly to nitrate.

The formation of the nitryl cation from oxoperoxonitrate(1–) has largely been overlooked, because nitryl is a powerful oxidant [$E^{\circ}(NO_2^+/{}^{\bullet}NO_2)$ = 1.6 V] that occurs in a solution of nitric and sulfuric acid used for the preparation of nitrophenols. As shown above, without a catalyst, formation of the nitryl cation is prevented by the large activation energy of heterolysis. Transition metal ions like Fe^{3+} and Cu^{2+} catalyze heterolysis and react with oxoperoxonitrate(1–) to form a potent nitrating agent with the reactivity of the nitryl cation, even when the metals are bound in proteins. We have recently shown that the Cu/Zn, Fe, and Mn forms of superoxide dismutase catalyze the direct nitration of a wide range of phenols by oxoperoxonitrate(1–) (*67*, *68*). This process appears to be mediated by the nitryl cation or a closely related species.

Oxoperoxonitrate(1–) directly oxidizes sulfhydryls by a second-order process (*25*), with an activation enthalpy of approximately 10 kcal/mol, as shown in Figure 5. A pathway involving a nitryl-like intermediate could be envisioned for the oxidation of sulfhydryls:

$$RSH + ONOOH \rightarrow RHS^+O^- + NO_2^- + H^+ \quad (6)$$

resulting initially in a sulfenic intermediate with sulfinic acid as the final product.

Thus, oxoperoxonitrate(1–) could cause toxic reactions in vivo by at least three mechanisms: (i) hydrogen ion-catalyzed formation of potent oxidants with the reactivities of hydroxyl radical and nitrogen dioxide, (ii) direct molecular reactions, as, for example, with sulfides (*26*) and sulfhydryl groups (*25*), and (iii) reaction with metal ions to form a potent nitrating agent resembling the nitryl cation.

Although decomposition of oxoperoxonitrate(1–) acid gives rise to a strong oxidant resembling the hydroxyl radical, the direct reactions of oxoperoxonitrate(1–) with cellular thiols, sulfides, or transition metal complexes may be of greater physiological significance. The high reactivity of the hydroxyl radical (*69*) limits its toxicity by decreasing its ability to reach critical sites. In contrast, oxoperoxonitrate is longer-lived and appears to be more selective. Calculations analogous to those for ozone (*70*) indicate that the hydronated form, which has a half-life of 0.15 s at 37 °C in water, lives long enough to reach a critical target within a lipid bilayer, or to diffuse through it. We

assumed above that the solvation energy of hydrogen oxoperoxonitrate is the same as that of hydrogen peroxide. The latter is soluble in a number of organic solvents, and it is likely therefore that hydrogen oxoperoxonitrate can enter a lipid bilayer and initiate lipid peroxidation, as has been experimentally observed (*24*). When hydrogen oxoperoxonitrate isomerizes and forms a transient strong oxidant, one could in principle scavenge this transient and thereby expect protection. However, scavenging results in the formation of nitrogen dioxide, which is toxic in itself. For example, we have recently shown that oxoperoxonitrate(1−) is highly bactericidal to *Escherichia coli*. Addition of DMSO to scavenge the intermediate *increased* the bactericidal killing, which was correlated with the enhanced production of nitrogen dioxide (*71*).

Conclusion

The isomerization of hydrogen oxoperoxonitrate to nitrate and a hydron does not proceed via a free hydroxyl radical or a nitryl cation. It seems likely that an intermediate that is structurally related to the transition state formed in the isomerization reaction of HOON=O to $HONO_2$ is responsible for oxidations that are kinetically first-order overall. This intermediate appears to be a hydrogen oxoperoxonitrate molecule in the trans configuration with a lengthened O–O bond and the terminal oxygen near the nitrogen. Undistorted oxoperoxonitrate(1−) reacts with bimolecular kinetics with thiols and thioethers and forms a strongly nitrating species in the presence of metal ions or complexes.

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Registry No. $ONOO^-$, 19059-14-4; $^{\bullet}NO$, 10102-43-9; OH^-, 14280-30-9; NO_2^+, 14522-82-8; $O_2^{\bullet-}$, 11062-77-4; ONOOH, 14691-52-2; $^{\bullet}NO_2$, 10102-44-0; glutathione, 70-18-8; cysteine, 52-90-4; dimethyl sulfoxide, 67-68-5; deoxyribose, 533-67-5.