

Formation of nitrous oxide from the reaction of peroxy-nitrite with sodium azide

Sarojini Padmaja,* Van Au and Stephen A. Madison*

Organic Division, Unilever Research, U.S., 45 River Road, Edgewater, New Jersey 07020, USA

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The reaction of peroxy-nitrite (oxoperoxonitrate) with azide ion (N_3^-), the residual starting material in the peroxy-nitrite preparation by the ozonolysis of azide, was investigated in phosphate and carbonate buffers. The observed rate constants (k_{obs}) for the decay of peroxy-nitrite at pH 4.5 in the presence of azide up to 30 mM are within experimental error identical to the self decomposition rate of peroxy-nitrite indicating that the reaction is zero order in azide concentration. However, when the concentration of azide is increased beyond 40 mM a slight increase in k_{obs} is noticed. From the dependence of k_{obs} on the concentration of azide the second-order rate constants for the reaction of peroxy-nitrite with azide are determined to be 3.5 and 1.2 $\text{M}^{-1} \text{s}^{-1}$ at pH 4.5 and 7.4 respectively. In the presence of added bicarbonate the reaction between peroxy-nitrite and azide is zero order in azide concentration. The reaction of peroxy-nitrite with azide led to the production of nitrous oxide (N_2O) in the absence and presence of bicarbonate as identified by GC-MS analysis of the reaction mixture. At a given pH and peroxy-nitrite concentration, the yield of N_2O increased linearly with an increase in the concentration of azide up to 100 mM and attained saturation beyond that. Under identical conditions, the yield of N_2O obtained in the absence of bicarbonate is 50% more compared to that obtained in the presence of bicarbonate. Based on kinetics and product studies, it is proposed that the reaction of peroxy-nitrite with azide involves a one-electron oxidation of N_3^- to azide radical (N_3^\cdot) by the activated form of HOONO (HOONO*). Also that the combination between N_3^\cdot and nitrogen dioxide (NO_2^\cdot) is rapid and leads to nitryl azide (N_3NO_2) as a transient intermediate and precursor for nitrous oxide (N_2O). In the presence of bicarbonate the peroxy-nitrite-carbon dioxide adduct (ONOOCO_2^-) or the carbonate radical anion ($\text{CO}_3^{\cdot-}$) is proposed as a one-electron oxidant towards N_3^- forming N_3^\cdot . The proposed mechanism for N_2O formation from nitryl azide is supported by high level *ab initio* calculations.

Introduction

Peroxy-nitrite (oxoperoxonitrate) is an important cytotoxic agent formed by the direct and rapid combination of nitric oxide and superoxide $\text{O}_2^{\cdot-}$.¹⁻³ It reacts with a number of biological targets such as DNA,⁴ lipids,⁵ sulfides,⁶ seleno compounds⁷⁻⁹ and nitrates tyrosine and other phenolic compounds.^{10,11} Peroxy-nitrite can isomerize to nitrate or decompose to nitrite and oxygen. Isomerization to nitrate is the major pathway in acidic media and in this pathway ONOOH produces a highly reactive oxidizing intermediate (HOONO*),^{12,13} which might account for the hydroxyl radical-like properties of peroxy-nitrite. The decomposition of peroxy-nitrite above pH 5.0 mainly produces nitrite and oxygen which is suggested to occur through the oxidation of ONOO⁻ by the activated intermediate.¹⁴ The decomposition pathways of peroxy-nitrite in phosphate buffers have been subject to numerous investigations recently^{12,14,15} and despite the large number of studies the mechanism underlying the decomposition of peroxy-nitrous acid to nitrate or the identity of the proposed activated intermediate of peroxy-nitrous acid (HOONO*) is not fully understood.

Recently it was reported that peroxy-nitrite rapidly reacts with CO_2 to form an adduct, ONOOCO_2^- ,^{16,17} with a rate constant of $3 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ at 25 °C. Studies by Lyman and Hurst¹⁸⁻²⁰ and Goldstein and Czapski²¹ showed that the decomposition of the ONOOCO_2^- adduct generates reactive intermediates capable of oxidizing organic and inorganic compounds. Thus it is evident that both HOONO and the ONOOCO_2^- adduct decompose through the intermediacy of strongly oxidizing intermediates.

During our peroxy-nitrite reaction studies we observed production of gaseous products from the decomposition of

peroxy-nitrite prepared by the ozonation of azide.²² This observation prompted us to undertake a detailed investigation on the kinetics and mechanistic aspects of the reaction of peroxy-nitrite with azide. We here report for the first time that the decomposition of peroxy-nitrite in the presence of sodium azide in phosphate and bicarbonate buffers led to the production of nitrous oxide as the major gaseous product in addition to nitrite and nitrate. Further, we observed low yields of oxygen from the decomposition of peroxy-nitrite (only in samples prepared by the ozonation of azide) in carbonate buffers. The kinetics, products and possible mechanistic pathways are discussed to account for these experimental observations.

Materials and methods

Preparation of peroxy-nitrite

Method 1. Peroxy-nitrite was prepared by the ozonolysis of sodium azide according to the method of Uppu *et al.*²² A Sander 200 ozonizer from Erwin Sander (Uetze-Eltze) was used to ozonize the azide solutions. The resulting peroxy-nitrite solution was purged with argon and freeze fractionated to yield typically 0.2–0.4 M peroxy-nitrite.

Method 2. Peroxy-nitrite was prepared by the reaction of isopentyl nitrite with H_2O_2 under alkaline conditions as described by Uppu and Pryor.²³

Identification and quantification of nitrous oxide

The reaction of peroxy-nitrite with azide was performed both in the presence and absence of bicarbonate in phosphate buffer

containing 0.1 mM EDTA. All buffers were freshly prepared by using deionized and degassed (purged with argon) water.

Nitrous oxide was detected using an HP 5890 series II gas chromatograph equipped with a Chrompack PoraPlot column (27.5 m × 0.32 mm × 10 μm ID) and a thermal conductivity detector at 25 °C with a helium flow rate of 50 mL min⁻¹. The N₂O peak was identified by comparison to an authentic sample of N₂O by retention time.²⁴ The reaction of peroxy-nitrite with azide was carried out in a sealed 22 mL vial (liquid volume is 18 mL) under argon saturated conditions. A 500 μL volume of gaseous products from the head space was analysed using a Pressure-Lock © syringe. Quantification of N₂O was carried out by determining the total amount of N₂O in the gas and aqueous phase using eqn. (1) where *N* is the total number

$$N = C_g (V_g + aV_l) \quad (1)$$

of moles of N₂O in the reaction mixture, *C_g* the concentration of N₂O in the gas phase, *V_g* the volume of the gas phase, *V_l* that of the liquid phase and *a* = 0.544 at 25 °C is the Bunsen adsorption coefficient of N₂O.^{24,25}

Identification and quantification of oxygen and nitrogen

The reaction of peroxy-nitrite with azide was performed in phosphate buffer both in the presence and absence of bicarbonate for quantification of N₂ and O₂ if any formed from the reaction. Solutions were saturated with helium since argon interferes with the GC detection of O₂.

Nitrogen and oxygen were detected using an HP 5890 series II gas chromatograph equipped with a Chrompack CP-Molsieve 5A Plot fused silica column (30.0 m × 0.7 mm × 15 μm film thickness) and a thermal conductivity detector at 25 °C with a helium flow rate of 50 mL min⁻¹. Peroxy-nitrite was decomposed in a sealed 22 mL vial under argon saturated conditions. As described for N₂O above, quantification of N₂ and O₂ was carried out by determining the total amount of N₂ and O₂ in the gas and aqueous phase using eqn. (1). In the latter, however, *C_g* equals the concentration of N₂ or O₂ in the gas phase, and *a* = 0.0146 for N₂ and 0.0289 for O₂ at 24 °C are the Bunsen adsorption coefficients.²⁶

Stopped-flow experiments

Stopped-flow kinetic studies were performed using an OLIS-RSM 1000 stopped flow instrument equipped with a 2 cm observation cell and a mixing time of less than 1 millisecond. All experiments were conducted under degassed conditions by purging the solutions with argon to remove CO₂ from the system. The kinetics of the reactions was followed by monitoring the decay of peroxy-nitrite anion absorption at 302 nm (pH > 6.0) or the decay of peroxy-nitrous acid at 260 or 350 nm (pH < 6.0). Reactions were carried out under pseudo-first-order conditions with respect to azide and the observed rate constants (*k_{obs}*) were extracted from a single-exponential fit to the absorbance vs. time curve. Data were analysed by the OLIS-RSM global fit software. The reaction of the peroxy-nitrite-CO₂ (ONOCO₂⁻) adduct with azide was studied by mixing peroxy-nitrite with an excess of bicarbonate so that all peroxy-nitrite anion is first converted into the adduct and subsequently reacts with azide. All experiments were conducted at room temperature (23 ± 1 °C) and the solution pH was measured at the stopped-flow outlet.

Computational methodology

The geometries and energies were calculated with density functional theory (DFT) using the Becke3LYP functional and 6-31+G(d) basis set contained within the GAUSSIAN 94 suite of programs.²⁷ All minima and transition state structures were fully optimized with the GAUSSIAN default convergence

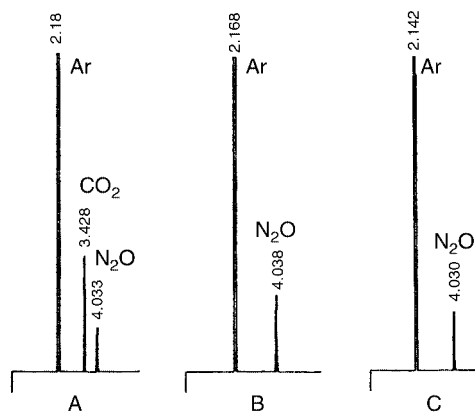


Fig. 1 GC Chromatogram of the reaction mixture of peroxy-nitrite (2 mM) and azide (100 mM) in 0.2 M phosphate buffer: (A) in the presence of bicarbonate, (B) in the absence of bicarbonate and (C) authentic N₂O. Retention times in min.

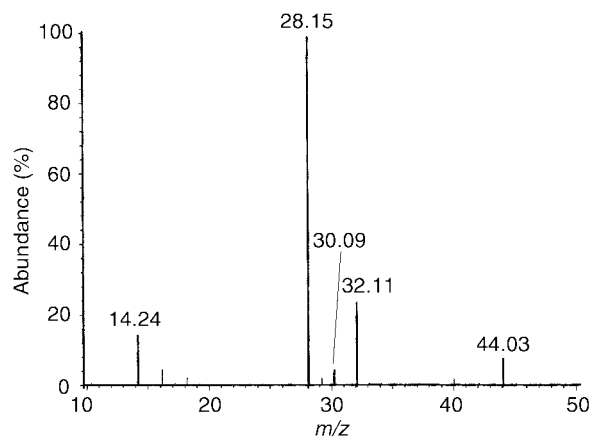


Fig. 2 Electron impact mass spectrum of the reaction mixture of peroxy-nitrite (2 mM) and azide (100 mM) without bicarbonate in 0.2 M phosphate buffer.

criteria for energies and wave functions and characterized by harmonic vibrational frequency analysis. Transition and ground state structures had one and zero imaginary frequencies, respectively. Intrinsic reaction coordinate (IRC) calculations based on the method of Gonzalez and Schlegel^{28,29} and as implemented in the GAUSSIAN 94 program were used to verify the path that links reactant and product *via* the transition state structure.

Results

Reaction of peroxy-nitrite with azide ion-product analysis

Decomposition of peroxy-nitrite prepared by the ozonation of sodium azide results in the liberation of gases in both phosphate and bicarbonate buffers while decomposition of peroxy-nitrite prepared by method 2 did not yield any measurable gaseous products under identical conditions. For the identification of gaseous products both head space GC and GC-MS analysis of the reaction mixture were performed. Fig. 1A and 1B show the GC chromatograms of the reaction mixture obtained from 2 mM peroxy-nitrite and 100 mM azide at pH 7.0 in the presence and absence of bicarbonate, respectively. Nitrous oxide was identified as the major gaseous product by comparing with the GC of an authentic sample with a retention time of 4.03 min (Fig. 1C). The presence of N₂O is further confirmed by mass spectral measurements. The electron impact mass spectrum of the reaction mixture of peroxy-nitrite with azide under degassed conditions (purged with N₂) displayed a peak with an *m/z* value at 44 and fragmentation at 30 which are signatures of N₂O (Fig. 2). Further, we did not observe CO₂

Table 1 Yield of gaseous products obtained from the reaction of 2 mM peroxyntirite with 60 mM azide at different pH and 25 °C

pH	Yield of N ₂ O/ μ mol	Yield of O ₂ / μ mol	Yield of N ₂ / μ mol
4.0	6.98	0.00	0.00
7.0	6.90	0.52	0.82

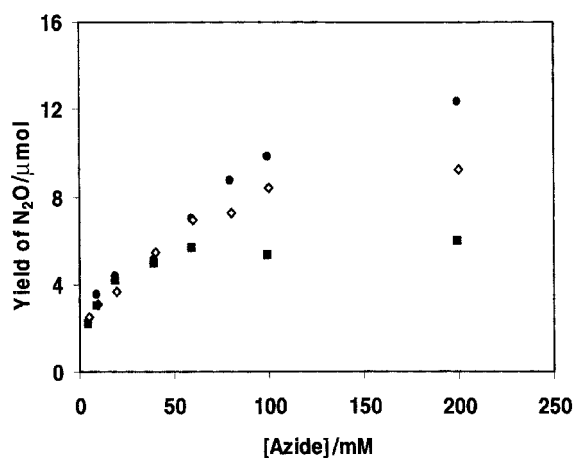


Fig. 3 Yield of N₂O as a function of the concentration of azide from the reaction of peroxyntirite with azide in 0.2 M buffer: (●) pH 4.0, (◇) pH 7.0 and (■) in presence of 12 mM CO₂ at pH 7.0. The concentration of peroxyntirite was 2 mM (36 μ mol) and that of azide varied from 0 to 400 mM. Nitrous oxide was analysed by GC and identified by comparison with the GC of authentic nitrous oxide.

by GC in the reaction of peroxyntirite without bicarbonate, but an m/z of 44 in the mass spectrum is observed. Thus it is confirmed that the m/z at 44 is due to N₂O. Fig. 3 shows the yield of N₂O obtained from the reaction of peroxyntirite with azide at pH 4.0 and 7.0 as a function of the azide concentration. The yield of N₂O at both pH 4.0 and 7.0 increased linearly with an increase in the concentration of azide and reached a plateau value at high azide concentrations. We also looked for other gaseous products such as N₂ and O₂ in the reaction of peroxyntirite with azide. The yields of N₂ (retention time = 5.3 min) and O₂ (retention time = 3.1 min) as determined by head space GC analysis is very low compared to the yield of N₂O (Table 1).

The reaction of the peroxyntirite-carbon dioxide adduct with azide was studied at pH 7.0 in presence of an excess of carbon dioxide in the reaction system. Fig. 3(■) shows the yield of N₂O obtained from the reaction of 2 mM peroxyntirite with varying amounts of azide in presence of 12 mM CO₂. Under identical conditions, the yield of nitrous oxide obtained in presence of CO₂ is 50% less than that observed in the absence of CO₂.

Effect of added nitrite ion

The effect of added sodium nitrite on the yield of nitrous oxide was studied both in the absence and presence of bicarbonate. The yield of N₂O as a function of azide was measured in the presence of equivalent amounts of nitrite to azide. Fig. 4 shows the yield of N₂O obtained as a function of azide concentration with 75 mM bicarbonate and varying amounts of nitrite. It is evident that nitrite did not influence the yield of N₂O up to 100 mM of azide and a slight increase is noted when the nitrite concentration is increased above 100 mM. In the absence of bicarbonate, nitrite did not influence the yield of N₂O obtained from the reaction of peroxyntirite with azide ranging from 0 to 300 mM (data not shown).

Kinetics of the reaction between peroxyntirite and azide

Peroxyntirite prepared by method 2 was used for the kinetic

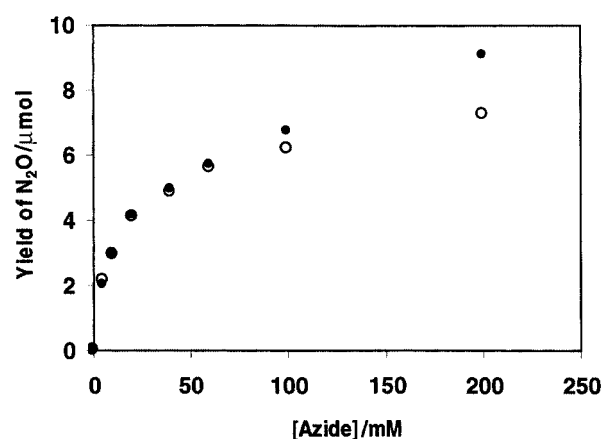


Fig. 4 Yield of N₂O as a function of the concentration of azide from the reaction of peroxyntirite with azide in 0.2 M phosphate buffer containing 75 mM bicarbonate: (○) in the absence of nitrite and (●) in the presence of equimolar concentrations of nitrite.

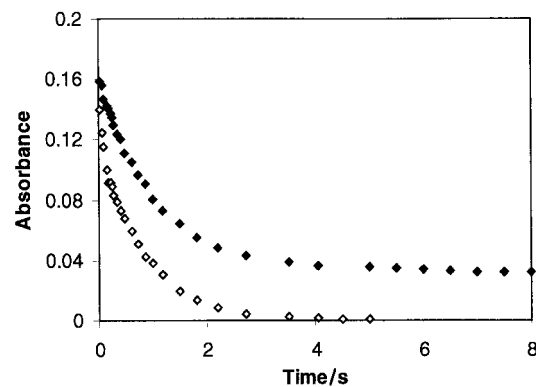


Fig. 5 Stopped-flow kinetics of peroxyntirous acid decay at 350 nm and pH 4.5 in presence of azide: (◆) without azide and (◇) in presence of 100 mM azide. The concentration of peroxyntirite was 0.4 mM. Reaction was initiated by stopped-flow mixing of degassed solutions of acetate buffer with or without azide containing 0.1 mM dtpa (dtpa = diethylenetriaminepentaacetic acid) and an alkaline solution of peroxyntirite.

studies of its reaction with azide ion. The decomposition of peroxyntirite was followed in the presence of various concentrations of azide at both pH 4.5 and 7.4 at 23 \pm 0.2 °C. Fig. 5 shows the decay of HOONO at pH 4.5 monitored at 350 nm in the absence and in the presence of 100 mM azide. The concentrations of azide ranging from 0 to 30 mM did not enhance the rate of decomposition of peroxyntirite. The observed rate constants (k_{obs}) were within experimental error identical to those of the self-decay of peroxyntirite indicating that the reaction is zero-order in azide concentration (see inset of Fig. 6). The rate law for the reaction between peroxyntirite and azide to account for the observed rate data can be expressed as in eqn. (2), where

$$-d[\text{HOONO}]_{\text{T}}/dt = k_1[\text{HOONO}][\text{Azide}]^0 \quad (2)$$

$[\text{HOONO}]_{\text{T}}$ is the sum of HOONO and ONOO⁻, and k_1 the rate constant for the self decomposition of peroxyntirite in presence of azide. From eqn. (2), k_{obs} for the disappearance of peroxyntirite is given by eqn. (3). When the concentration of

$$k_{\text{obs}} = k_1[\text{H}^+]/(K_1 + [\text{H}^+]) \quad (3)$$

azide was increased beyond 40 mM the k_{obs} increased linearly with increasing concentrations of azide indicating that at high concentrations the reaction is bimolecular. For the bimolecular reaction between ground state peroxyntirite and azide the rate law for the disappearance of HOONO can be expressed as in eqn. (4). From eqn. (4), the k_{obs} for the overall disappearance

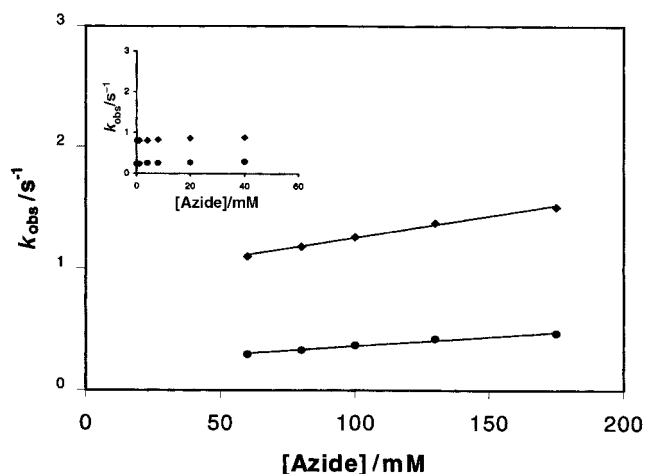


Fig. 6 Plot of observed rate constant (k_{obs}) as a function of the concentration of azide: (◆) at pH 4.5 and 23 °C, (●) pH 7.4 and 23 °C. The concentration of peroxyntirite was 0.4 mM. The inset shows the plot of k_{obs} vs. [azide] for the low concentrations range of azide.

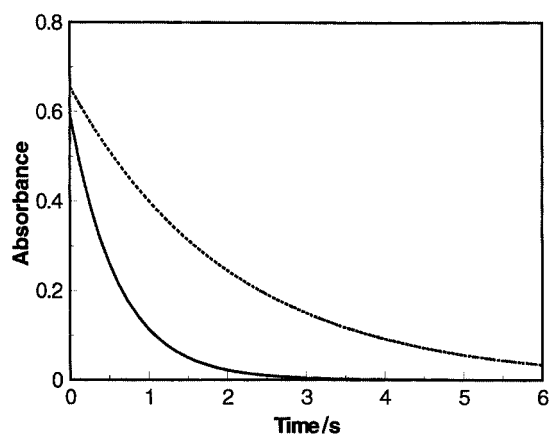


Fig. 7 Stopped-flow kinetics of peroxyntirite decay at 302 nm and pH 7.0 in presence of azide: (---) without bicarbonate and (—) in presence of 5 mM bicarbonate. The concentration of peroxyntirite was 0.2 mM and that of azide was 20 mM. Reaction was initiated by stopped-flow mixing of degassed solutions of azide with or without bicarbonate in phosphate buffer containing 0.1 mM dtpa and an alkaline solution of peroxyntirite.

of peroxyntirite at high concentrations of azide is given by eqn. (5), where k_2 is the bimolecular rate constant for the reaction

$$-d[\text{HOONO}]_T/dt = k_2[\text{HOONO}][\text{Azide}] \quad (4)$$

$$k_{\text{obs}} = \frac{k_1[\text{H}^+]}{(K_1 + [\text{H}^+])} + \frac{k_2[\text{H}^+]}{(K_1 + [\text{H}^+])}[\text{Azide}] \quad (5)$$

between ground state peroxyntirite and azide. Fig. 6 shows the plot of k_{obs} vs. [Azide], the slopes of which gave bimolecular rate constants (k_2) of 3.5 and 1.2 $\text{M}^{-1} \text{s}^{-1}$ at pH 4.5 and 7.4 respectively for the reaction between HOONO and azide.

The reaction of the peroxyntirite- CO_2 adduct with azide was studied by mixing peroxyntirite with an excess of bicarbonate to ensure that all the peroxyntirite had converted into the adduct which subsequently reacts with azide. Fig. 7 shows the decomposition of peroxyntirite at 302 nm in the presence of azide with and without bicarbonate at pH 7.4. The k_{obs} for peroxyntirite decomposition is 0.24 s^{-1} in the absence of bicarbonate and 10 s^{-1} in presence of 5 mM bicarbonate. In either case, the rate of the reaction was independent of the concentrations of azide. The acceleration of the decay of peroxyntirite in the presence of bicarbonate is due to the rapid reaction of peroxyntirite anion with carbon dioxide.

Table 2 Energies (au) for ground and transition state structures

Compound	Becke3LYP 6-31+G(d) energy/au	ZPE ^a
Nitryl azide	-369.267196	15.1(0)
TS-1	-369.236175	14.7(1)
Cyclic intermediate A	-369.251216	15.4(0)
TS-2	-369.240639	14.2(1)
Nitrous oxide	-184.668295	6.9(0)

^a Zero point energy (kcal mol^{-1}), unscaled. The number of imaginary vibrational frequencies is given in parentheses.

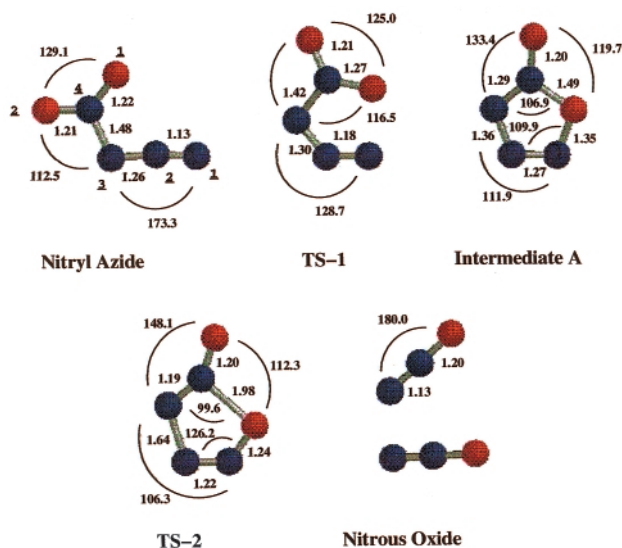


Fig. 8 Becke3LYP 6-31+G(d)-optimized structures in the potential energy surface for nitrous oxide formation. Bond lengths in Å, angles in °.

Ab initio calculations

High level *ab initio* calculations with the 6-31+G (d) basis set and the Becke3LYP HF/DFT hybrid functional were used to support an intramolecular process that involves nitryl azide, N_4O_2 , as a precursor of nitrous oxide. Transition and ground state structures were located for the processes that convert nitryl azide into the cyclic five-membered intermediate A and this cyclic intermediate into two molecules of nitrous oxide as shown in Fig. 8. Energies for the optimized structures are in Table 2. In the first step a calculated activation barrier of 19.5 kcal mol^{-1} was determined for the formation of cyclic intermediate A from nitryl azide. The imaginary frequency for this TS structure was found at 372.61 cm^{-1} and corresponds to the motion that eventually results in bond formation between the terminal azido nitrogen and oxygen of the nitro moiety. Verification that this TS structure links nitryl azide and the cyclic intermediate A was obtained from the results of an IRC calculation at the B3LYP 6-31+G(d) level. The IRC method is used to determine a reasonable potential energy surface by locating the two steepest descent paths leading from the TS down to the reactant and product local minima, respectively. In the forward (product) direction of the IRC calculation the TS structure, TS-1, leads to a structure at an IRC length of 2.80 $\text{amu}^{1/2} \text{ bohr}$ that is cyclic in nature and where the N1-O1 atomic distance is 1.36 Å and the angle O1-N1-N2 equals 109.9°. For reference, cyclic intermediate A is respectively 1.35 Å and 109.4° at these measured positions. In the reverse (reactant) direction the TS structure clearly links to nitryl azide as a similar structure with an N1-O1 distance of 3.06 Å and N1-N2-N3 of 177.3° is calculated at an IRC length of 6.99 $\text{amu}^{1/2} \text{ bohr}$.

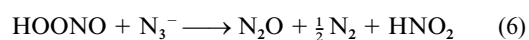
The putative cyclic intermediate A lies approximately 10 kcal mol^{-1} higher in energy than nitryl azide and only 6.7 kcal mol^{-1}

separates this intermediate and the TS structure, TS-2, that leads to the formation of two molecules of nitrous oxide; TS-2 involves substantial stretching of both the N2–N3 and N1–O1 bonds and is clearly part of the potential energy surface that connects both intermediate A and nitrous oxide. Thus, the IRC calculation along the TS-2 to intermediate A pathway results in an optimized structure at an IRC length of 1.998 where the N1–O1 bond distance is 1.62 Å (intermediate A N1–O1 1.49 Å). Along the nitrous oxide path TS-2 links to nitrous oxide. At the IRC length of 5.00 the N1–O1 and N2–N3 atomic distances are 2.58 and 2.41 Å, respectively. Further, the N3–N4–O2 and N2–N1–O1 angles are 174.2 and 169.0°, respectively and near that of linear nitrous oxide. Formation of nitrous oxide is highly exothermic and nearly 44 kcal mol⁻¹ lower in energy than nitryl azide.

Discussion

Activated intermediate of peroxy-nitrous acid as a one-electron oxidant

The present study reports a novel finding that peroxy-nitrite reacts with azide, the residual starting material in the peroxy-nitrite preparation by the ozonation of azide,²² to produce nitrous oxide as the major gaseous product. Previous studies report nitrite, nitrate and oxygen as products from the decomposition of peroxy-nitrite in phosphate buffers.¹⁴ The observed bimolecular rate constant for the reaction between peroxy-nitrite and azide at pH 4.5 is slow ($k_2 = 3.5 \text{ M}^{-1} \text{ s}^{-1}$) and decreased with increasing pH. The decrease in rate constant with pH suggests that HOONO rather than ONOO⁻ ($\text{p}K_a$ of HOONO = 6.8) is the reactive species involved in the bimolecular reaction with azide. Therefore, azide cannot directly compete for peroxy-nitrite in its bimolecular reactions with substrates, but could diminish the oxidation yield of reactions dependent on HOONO* by competing with reacting targets. At low azide concentrations the k_{obs} for the decomposition of peroxy-nitrite is independent of azide concentration. The above observations suggest that peroxy-nitrite reacts with azide by mixed direct and indirect oxidation mechanisms. In this respect, the reaction is similar to the oxidations of ascorbate,³⁰ methionine,³¹ tryptophan,³² ferrocyanide,³³ selenomethionine⁷ and 2-keto-4-thiomethylbutanoic acid³¹ by peroxy-nitrite where a zero-order and first-order dependency of k_{obs} on the substrate is observed. For ascorbate,³⁰ tryptophan³² and methionine³¹ the plots of k_{obs} vs. [substrate] show two regions: one linear and one curved. However, in the case of azide no curvature is observed at low substrate concentrations which is similar to that observed for DMPO³⁴ (5,5-dimethyl-1-pyrroline *N*-oxide). Goldstein *et al.*¹³ reported that in the presence of an inefficient scavenger of HOONO* the plot of k_{obs} vs. [substrate] will yield a straight line with no curvature. In the indirect oxidation pathway since the reaction is zero order in azide concentration the activated intermediate of peroxy-nitrous acid (HOONO* or some HO radical-like species) determines the reaction with azide. Peroxy-nitrite can react through one and two-electron oxidation mechanisms depending on the nature of the substrate.^{7,31,33} A direct oxidation of azide by an oxygen transfer mechanism is possible which could produce one equivalent of N₂O and 0.5 equivalent of N₂ as gaseous products as shown in eqn. (6).



Our product studies showed that nitrous oxide accounts for 90% of the gaseous products obtained in the reaction of peroxy-nitrite at pH 7.0 with approximately 10% of N₂ (Table 1) and at pH 4.0 N₂O is the only gaseous product obtained. This rules out the possibility of a direct oxygen transfer mechanism by HOONO. Also, the plot of the yield of N₂O vs. [N₃⁻] attained

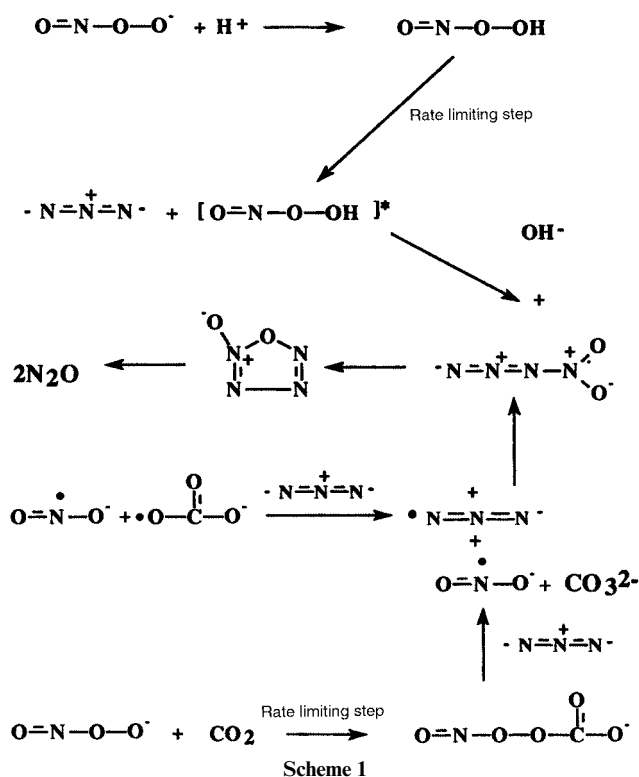
saturation at high azide concentrations. If the direct bimolecular reaction of HOONO with N₃⁻ resulted in the formation of N₂O one would expect an increase in N₂O yield with increase in [N₃⁻] at high concentrations. Further, in the bimolecular reactions of HOONO with substrates such as methionine,³¹ selenomethionine⁷ and threonylmethionine³⁵ a nearly 100% yield of the oxidation product is obtained while in the oxidation of azide the maximum yield of N₂O obtained is 16%. This clearly suggests that N₂O is not formed in the bimolecular reaction of HOONO with azide. Therefore, we propose that the reaction of peroxy-nitrite with azide occurs through a one-electron oxidation of azide by the activated intermediate (HOONO*) to generate azide radical. On analogy with the rapid reaction of [•]N₃ with [•]NO forming N₄O ($k = 4.4 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$),³⁶ we propose that the reaction of the resulting azide radical with nitrite radical will be rapid and leads to the unstable intermediate nitryl azide (N₄O₂), which subsequently decomposes to N₂O. The existence of these nitrogen oxides (N₄O, N₄O₂) has been documented^{37–39} and further it was shown that N₂O is the only gaseous product in the decomposition of nitryl azide.⁴⁰ The formation of nitryl azide from the reaction of azide and nitryl cation⁴⁰ and its structural elucidation by IR and Raman studies have also been reported.³⁸ The decomposition of nitryl azide to nitrous oxide requires an oxygen transfer from the nitro group to the azido group. High level *ab initio* calculations do indeed support a possible intramolecular process that involves nitryl azide as a precursor for N₂O (Fig. 8). The complete proposed mechanistic pathway leading to the formation of N₂O from the reaction of peroxy-nitrite with azide is shown in Scheme 1.

The plot of the yield of N₂O vs. concentration of azide (Fig. 3) shows saturation at concentrations higher than 100 mM and a 16% maximum yield of N₂O relative to peroxy-nitrite is obtained at pH 4.0 and 400 mM concentration of azide. Goldstein and Czapski⁴¹ suggested that about 60% of HOONO isomerizes directly to nitrate, whereas 40% is converted into HOONO* which subsequently oxidizes the substrate. Thus, the maximum yield of one-electron oxidation products will not exceed 40%. The fact that concentrations of azide such as 400 mM could trap only 16% of HOONO* suggests that azide is a poor scavenger for HOONO*.

Peroxy-nitrite–carbon dioxide adduct as a one-electron oxidant

The reaction of peroxy-nitrite with azide in the presence of bicarbonate displayed an overall second-order behaviour, first-order in peroxy-nitrite, first-order in bicarbonate and zero order in azide concentration. This suggests that the reaction between ONOO⁻ and CO₂ to form the ONOOCO₂⁻ adduct is rate limiting. Thus in the presence of bicarbonate the reaction will proceed through the intermediacy of the ONOOCO₂⁻ adduct or intermediates derived from it. It has been reported that ONOOCO₂⁻ and its derivatives are capable of oxidizing organic and inorganic compounds whose reduction potentials are 1.3 V²⁰ or lower.

The production of N₂O from the reaction of peroxy-nitrite with azide in the presence of bicarbonate is explained by a one-electron oxidation of N₃⁻ to [•]N₃ by ONOOCO₂⁻ adduct or CO₃^{•-}. The azide radical will be converted into nitryl azide and subsequently to N₂O as shown in Scheme 1. Interestingly, the yield of nitrous oxide obtained in carbonate buffer is 50% less than in phosphate buffer under identical conditions. The lower nitrous oxide yield in carbonate buffer may reflect the efficient adduct self-decomposition to nitrate and carbon dioxide that competes with the azide reaction. Addition of nitrite ion had no effect on the yield of N₂O in the presence of bicarbonate. This is as expected because nitrite cannot compete with azide ion for the reactive intermediates given the rate constants for CO₃^{•-} with azide⁴² and nitrite⁴³ are 2.2×10^7 and $6.6 \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$ respectively.



Conclusion

We have demonstrated that peroxynitrite reacts with azide in a first-order reaction producing nitrous oxide. In phosphate buffer, peroxynitrite-mediated oxidation of azide is facilitated by the activated intermediate of peroxynitrous acid (HO-ONO*) through a one-electron oxidation mechanism that forms nitryl azide as a transient intermediate. The proposed mechanism for N₂O formation from nitryl azide is supported by high level *ab initio* calculations. In carbonate buffer, the peroxynitrite-carbon dioxide adduct is proposed as the reactive species responsible for the one-electron oxidation of azide to azide radical and subsequently to nitryl azide.

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