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Formation of nitrous oxide from the reaction of peroxynitrite with sodium azide

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The reaction of peroxynitrite (oxoperoxonitrate) with azide ion (N_3^-), the residual starting material in the peroxynitrite preparation by the ozonolysis of azide, was investigated in phosphate and carbonate buffers. The observed rate constants (k_{obs}) for the decay of peroxynitrite at pH 4.5 in the presence of azide up to 30 mM are within experimental error identical to the self decomposition rate of peroxynitrite 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 peroxynitrite with azide are determined to be 3.5 and 1.2 M^{-1} s^{-1} at pH 4.5 and 7.4 respectively. In the presence of added bicarbonate the reaction between peroxynitrite and azide is zero order in azide concentration. The reaction of peroxynitrite 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 peroxynitrite 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 peroxynitrite with azide involves a one-electron oxidation of N_3^- to azide radical ($N_3^{\bullet}$) by the activated form of HOONO (HOONO*). Also that the combination between $N_3^{\bullet}$ and nitrogen dioxide ($NO_2^{\bullet}$) 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 peroxynitrite–carbon dioxide adduct ($ONOOCO_2^-$) or the carbonate radical anion ($CO_3^{\bullet -}$) is proposed as a one-electron oxidant towards N_3^- forming $N_3^{\bullet}$. The proposed mechanism for N_2O formation from nitryl azide is supported by high level *ab initio* calculations.

Introduction

Peroxynitrite (oxoperoxonitrate) is an important cytotoxic agent formed by the direct and rapid combination of nitric oxide and superoxide $O_2^{-\bullet}$.[1–3] It reacts with a number of biological targets such as DNA,[4] lipids,[5] sulfides,[6] seleno compounds[7–9] and nitrates tyrosine and other phenolic compounds.[10,11] Peroxynitrite 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 peroxynitrite. The decomposition of peroxynitrite 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 peroxynitrite 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 peroxynitrous acid to nitrate or the identity of the proposed activated intermediate of peroxynitrous acid (HOONO*) is not fully understood.

Recently it was reported that peroxynitrite rapidly reacts with CO_2 to form an adduct, $ONOOCO_2^-$,[16,17] with a rate constant of 3×10^4 M^{-1} s^{-1} at 25 °C. Studies by Lymar and Hurst[18–20] and Goldstein and Czapski[21] 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 peroxynitrite reaction studies we observed production of gaseous products from the decomposition of peroxynitrite 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 peroxynitrite with azide. We here report for the first time that the decomposition of peroxynitrite 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 peroxynitrite (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 peroxynitrite

Method 1. Peroxynitrite 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 peroxynitrite solution was purged with argon and freeze fractionated to yield typically 0.2–0.4 M peroxynitrite.

Method 2. Peroxynitrite 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 peroxynitrite 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 N_2O peak was identified by comparison to an authentic sample of N_2O by retention time.[24] The reaction of peroxynitrite 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 N_2O was carried out by determining the total amount of N_2O in the gas and aqueous phase using eqn. (1) where N is the total number

$$N = C_g (V_g + \alpha V_l) \quad (1)$$

of moles of N_2O in the reaction mixture, C_g the concentration of N_2O in the gas phase, V_g the volume of the gas phase, V_l that of the liquid phase and $\alpha = 0.544$ at 25 °C is the Bunsen adsorption coefficient of N_2O.[24,25]

Identification and quantification of oxygen and nitrogen

The reaction of peroxynitrite with azide was performed in phosphate buffer both in the presence and absence of bicarbonate for quantification of N_2 and O_2 if any formed from the reaction. Solutions were saturated with helium since argon interferes with the GC detection of O_2.

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}. Peroxynitrite was decomposed in a sealed 22 mL vial under argon saturated conditions. As described for N_2O above, quantification of N_2 and O_2 was carried out by determining the total amount of N_2 and O_2 in the gas and aqueous phase using eqn. (1). In the latter, however, C_g equals the concentration of N_2 or O_2 in the gas phase, and $\alpha = 0.0146$ for N_2 and 0.0289 for O_2 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 CO_2 from the system. The kinetics of the reactions was followed by monitoring the decay of peroxynitrite anion absorption at 302 nm (pH > 6.0) or the decay of peroxynitrous 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 peroxynitrite–CO_2 ($ONOOCO_2^-$) adduct with azide was studied by mixing peroxynitrite with an excess of bicarbonate so that all peroxynitrite 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 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.

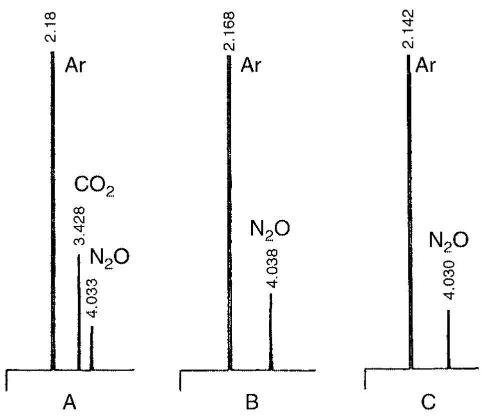


Fig. 1 GC Chromatogram of the reaction mixture of peroxynitrite (2 mM) and azide (100 mM) in 0.2 M phosphate buffer: (A) in the presence of bicarbonate, (B) in the absence of bicarbonate and (C) authentic N_2O. Retention times in min.

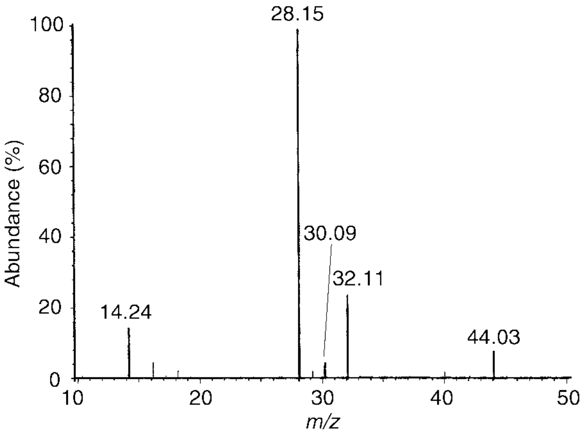


Fig. 2 Electron impact mass spectrum of the reaction mixture of peroxynitrite (2 mM) and azide (100 mM) without bicarbonate in 0.2 M phosphate buffer.

Results

Reaction of peroxynitrite with azide ion-product analysis

Decomposition of peroxynitrite prepared by the ozonation of sodium azide results in the liberation of gases in both phosphate and bicarbonate buffers while decomposition of peroxynitrite 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 peroxynitrite 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 N_2O is further confirmed by mass spectral measurements. The electron impact mass spectrum of the reaction mixture of peroxynitrite with azide under degassed conditions (purged with N_2) displayed a peak with an *m*/*z* value at 44 and fragmentation at 30 which are signatures of N_2O (Fig. 2). Further, we did not observe CO_2

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

pH	Yield of N_2O/μmol	Yield of O_2/μmol	Yield of N_2/μmol
4.0	6.98	0.00	0.00
7.0	6.90	0.52	0.82

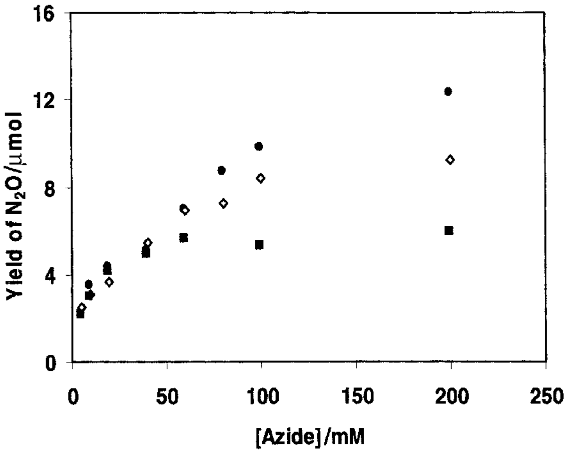


Fig. 3 Yield of N_2O as a function of the concentration of azide from the reaction of peroxynitrite with azide in 0.2 M buffer: (●) pH 4.0, (◊) pH 7.0 and (■) in presence of 12 mM CO_2 at pH 7.0. The concentration of peroxynitrite was 2 mM (36 μmol) and that of azide varied from 0 to 400 mM. Nitrous oxide was analysed by GC and identified by comparison with the GC of authentic nitrous oxide.

by GC in the reaction of peroxynitrite 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 N_2O. Fig. 3 shows the yield of N_2O obtained from the reaction of peroxynitrite with azide at pH 4.0 and 7.0 as a function of the azide concentration. The yield of N_2O 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 N_2 and O_2 in the reaction of peroxynitrite with azide. The yields of N_2 (retention time = 5.3 min) and O_2 (retention time = 3.1 min) as determined by head space GC analysis is very low compared to the yield of N_2O (Table 1).

The reaction of the peroxynitrite–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 N_2O obtained from the reaction of 2 mM peroxynitrite with varying amounts of azide in presence of 12 mM CO_2. Under identical conditions, the yield of nitrous oxide obtained in presence of CO_2 is 50% less than that observed in the absence of CO_2.

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 N_2O as a function of azide was measured in the presence of equivalent amounts of nitrite to azide. Fig. 4 shows the yield of N_2O 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 N_2O 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 N_2O obtained from the reaction of peroxynitrite with azide ranging from 0 to 300 mM (data not shown).

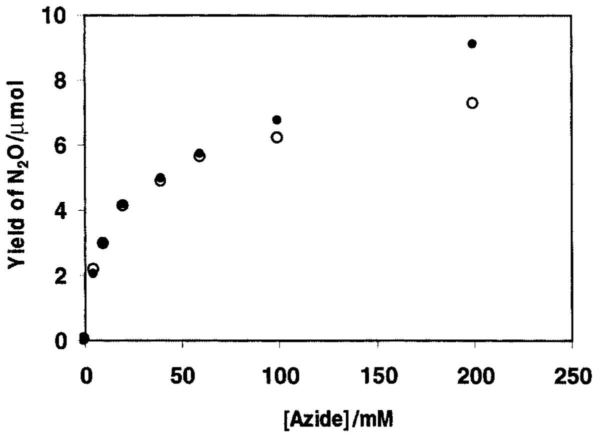


Fig. 4 Yield of N_2O as a function of the concentration of azide from the reaction of peroxynitrite with azide in 0.2 M phosphate buffer containing 75 mM bicarbonate: (○) in the absence of nitrite and (●) in the presence of equimolar concentrations of nitrite.

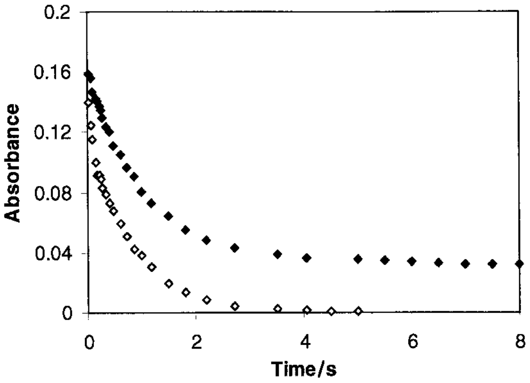


Fig. 5 Stopped-flow kinetics of peroxynitrous acid decay at 350 nm and pH 4.5 in presence of azide: (◆) without azide and (◇) in presence of 100 mM azide. The concentration of peroxynitrite was 0.4 mM. Reaction was initiated by stopped-flow mixing of degassed solutions of acetate buffer with or without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

Kinetics of the reaction between peroxynitrite and azide

Peroxynitrite prepared by method 2 was used for the kinetic studies of its reaction with azide ion. The decomposition of peroxynitrite was followed in the presence of various concentrations of azide at both pH 4.5 and 7.4 at 23 ± 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 peroxynitrite. The observed rate constants (k_{obs}) were within experimental error identical to those of the self-decay of peroxynitrite indicating that the reaction is zero-order in azide concentration (see inset of Fig. 6). The rate law for the reaction between peroxynitrite and azide to account for the observed rate data can be expressed as in eqn. (2), where

$$-d[HOONO]_T/dt = k_1[HOONO][Azide]^0 \qquad (2)$$

$[HOONO]_T$ is the sum of HOONO and $ONOO^-$, and k_1 the rate constant for the self decomposition of peroxynitrite in presence of azide. From eqn. (2), k_{obs} for the disappearance of peroxynitrite is given by eqn. (3). When the concentration of

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

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 peroxynitrite 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

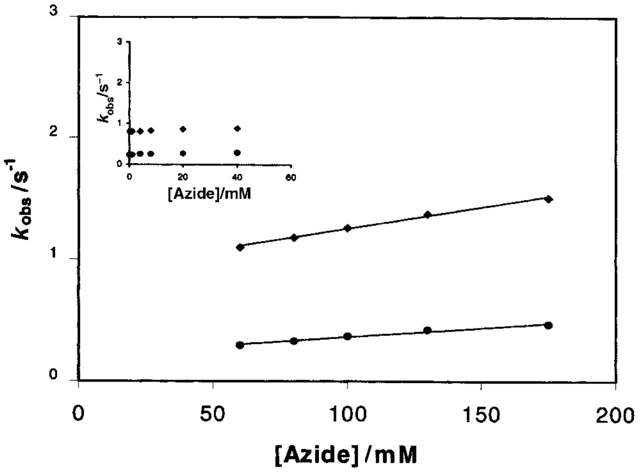


Fig. 6 Plot of observed rate constant (k_{obs}) as a function of the concentration of azide: (◆) at pH 4.5 and 23 °C, (●) pH 7.4 and 23 °C. The concentration of peroxynitrite was 0.4 mM. The inset shows the plot of k_{obs} *vs.* [azide] for the low concentrations range of azide.

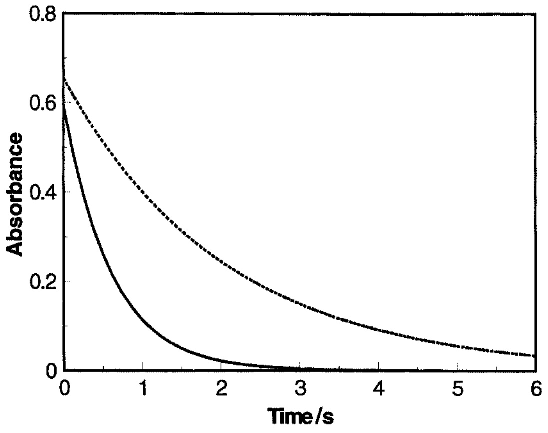


Fig. 7 Stopped-flow kinetics of peroxynitrite decay at 302 nm and pH 7.0 in presence of azide: (---) without bicarbonate and (——) in presence of 5 mM bicarbonate. The concentration of peroxynitrite was 0.2 mM and that of azide was 20 mM. Reaction was initiated by stopped-flow mixing of degassed solutions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

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

$$-\mathrm{d}[\mathrm{HOONO}]_{\mathrm{T}}/\mathrm{d}t = k_2[\mathrm{HOONO}][\mathrm{Azide}] \qquad (4)$$

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

between ground state peroxynitrite 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 M^{-1} s^{-1} at pH 4.5 and 7.4 respectively for the reaction between HOONO and azide.

The reaction of the peroxynitrite–CO_2 adduct with azide was studied by mixing peroxynitrite with an excess of bicarbonate to ensure that all the peroxynitrite had converted into the adduct which subsequently reacts with azide. Fig. 7 shows the decomposition of peroxynitrite at 302 nm in the presence of azide with and without bicarbonate at pH 7.4. The k_{obs} for peroxynitrite 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 peroxynitrite in the presence of bicarbonate is due to the rapid reaction of peroxynitrite 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.

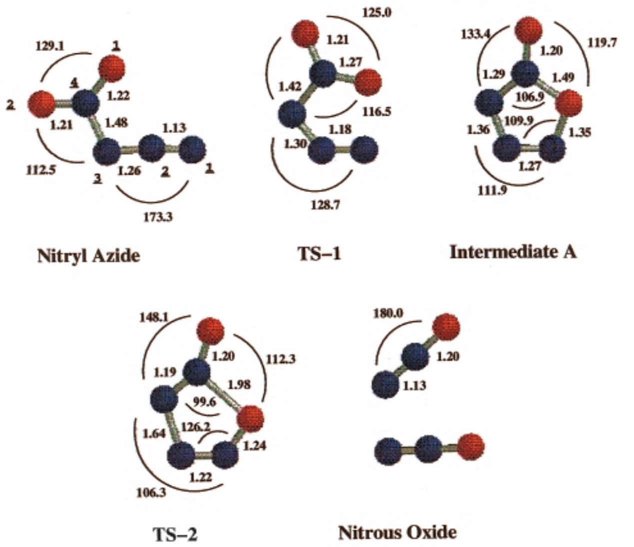


Fig. 8 Becke3LYP 6-31+G(d)-optimized structures in the potential energy surface for nitrous oxide formation. Bond lengths in Å, angles in °.

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 amu$^{1/2}$ 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 amu$^{1/2}$ 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 peroxynitrous acid as a one-electron oxidant

The present study reports a novel finding that peroxynitrite reacts with azide, the residual starting material in the peroxynitrite 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 peroxynitrite in phosphate buffers.[14] The observed bimolecular rate constant for the reaction between peroxynitrite and azide at pH 4.5 is slow ($k_2 = 3.5\ M^{-1}\ s^{-1}$) and decreased with increasing pH. The decrease in rate constant with pH suggests that HOONO rather than $ONOO^-$ (pK_a of HOONO = 6.8) is the reactive species involved in the bimolecular reaction with azide. Therefore, azide cannot directly compete for peroxynitrite 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 peroxynitrite is independent of azide concentration. The above observations suggest that peroxynitrite 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] selenomethionine[7] and 2-keto-4-thiomethylbutanoic acid[31] by peroxynitrite where a zero-order and first-order dependency of k_{obs} on the substrate is observed. For ascorbate,[30] tryptophan[32] and methionine[31] 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 DMPO[34] (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 peroxynitrous acid (HOONO* or some HO radical-like species) determines the reaction with azide. Peroxynitrite 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 N_2O and 0.5 equivalent of N_2 as gaseous products as shown in eqn. (6).

$$HOONO + N_3^- \longrightarrow N_2O + \tfrac{1}{2} N_2 + HNO_2 \qquad (6)$$

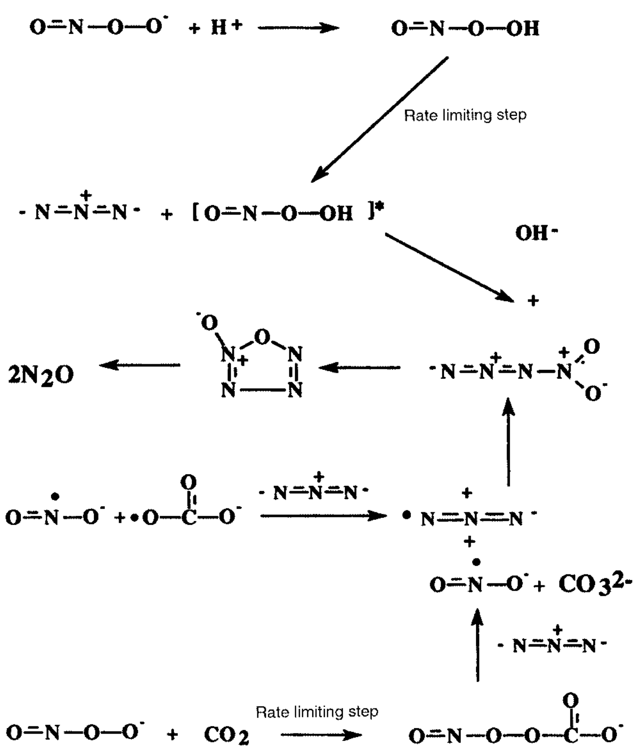
Our product studies showed that nitrous oxide accounts for 90% of the gaseous products obtained in the reaction of peroxynitrite at pH 7.0 with approximately 10% of N_2 (Table 1) and at pH 4.0 N_2O is the only gaseous product obtained. This rules out the possibility of a direct oxygen transfer mechanism by HOONO. Also, the plot of the yield of N_2O *vs.* $[N_3^-]$ attained saturation at high azide concentrations. If the direct bimolecular reaction of HOONO with N_3^- resulted in the formation of N_2O one would expect an increase in N_2O yield with increase in $[N_3^-]$ at high concentrations. Further, in the bimolecular reactions of HOONO with substrates such as methionine,[31] selenomethionine[7] and threonylmethionine[35] a nearly 100% yield of the oxidation product is obtained while in the oxidation of azide the maximum yield of N_2O obtained is 16%. This clearly suggests that N_2O is not formed in the bimolecular reaction of HOONO with azide. Therefore, we propose that the reaction of peroxynitrite 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 $^{\bullet}N_3$ with $^{\bullet}NO$ forming N_4O ($k = 4.4 \times 10^9\ M^{-1}\ s^{-1}$),[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 (N_4O_2), which subsequently decomposes to N_2O. The existence of these nitrogen oxides (N_4O, N_4O_2) has been documented[37–39] and further it was shown that N_2O is the only gaseous product in the decomposition of nitryl azide.[40] The formation of nitryl azide from the reaction of azide and nitryl cation[40] 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 N_2O (Fig. 8). The complete proposed mechanistic pathway leading to the formation of N_2O from the reaction of peroxynitrite with azide is shown in Scheme 1.

The plot of the yield of N_2O *vs.* concentration of azide (Fig. 3) shows saturation at concentrations higher than 100 mM and a 16% maximum yield of N_2O relative to peroxynitrite is obtained at pH 4.0 and 400 mM concentration of azide. Goldstein and Czapski[41] 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*.

Peroxynitrite–carbon dioxide adduct as a one-electron oxidant

The reaction of peroxynitrite with azide in the presence of bicarbonate displayed an overall second-order behaviour, first-order in peroxynitrite, first-order in bicarbonate and zero order in azide concentration. This suggests that the reaction between $ONOO^-$ and CO_2 to form the $ONOOCO_2^-$ adduct is rate limiting. Thus in the presence of bicarbonate the reaction will proceed through the intermediacy of the $ONOOCO_2^-$ adduct or intermediates derived from it. It has been reported that $ONOOCO_2^-$ and its derivatives are capable of oxidizing organic and inorganic compounds whose reduction potentials are 1.3 V[20] or lower.

The production of N_2O from the reaction of peroxynitrite with azide in the presence of bicarbonate is explained by a one-electron oxidation of N_3^- to $^{\bullet}N_3$ by $ONOOCO_2^-$ adduct or $CO_3^{\bullet -}$. The azide radical will be converted into nitryl azide and subsequently to N_2O 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 N_2O 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 $CO_3^{\bullet -}$ with azide[42] and nitrite[43] are 2.2×10^7 and $6.6 \times 10^5\ M^{-1}\ s^{-1}$ respectively.



Scheme 1

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 N_2O 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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