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Review

Relationships between nitric oxide, nitroxyl ion, nitrosonium cation and peroxynitrite

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Abstract

This review is concerned mainly with the three redox-related, but chemically distinct, species NO^-, NO• and NO^+, with greatest emphasis being placed on the chemistry and biology of the nitroxyl ion. Biochemical routes for the formation of nitroxyl ion and methods for showing the intermediacy of this species are discussed, together with chemical methods for generating nitroxyl ion in solution. Reactions of nitroxyl ion with NO•, thiols, iron centres in haem and with dioxygen are reviewed The significance of the reaction between NO^- and dioxygen as a source of peroxynitrite is assessed, and attention drawn to the possible significance of the spin state of the nitroxyl ion in this context. The biological significance of nitrosation and the importance of *S*-nitrosothiols and certain metal nitrosyl complexes as carriers of NO^+ at physiological pH is stressed. Some features in the chemistry of peroxynitrite are noted.

Keywords: Nitric oxide; Nitroxyl anion; Peroxynitrite

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1. Introduction

This review deals with the relationships between the nitroxyl anion (NO^-, oxonitrate(1−)), nitric oxide ($NO^\bullet$, nitrogen monoxide), the nitrosonium cation (NO^+, nitrosyl cation) and peroxynitrite ($ONOO^-$, oxoperoxonitrate(1−)), in which the nitrogen atom has formal positive oxidation states of I, II, III and III, respectively. The immense importance of nitric oxide in the life sciences and medicine needs no elaboration. Peroxynitrite has also attracted considerable attention as a species through which the cytotoxic effects of nitric oxide may be manifested under oxidising conditions, as peroxynitrite can be formed by reaction between nitric oxide and superoxide ion (Eq. 1) [1]. It should be noted, however, that Fukuto and Ignarro have recently suggested that the evidence supporting the presence of peroxynitrite in biological systems is indirect and potentially ambiguous and that other mechanisms may explain NO-mediated toxicity under oxidative conditions in vivo [2].

The species nitroxyl ion and the nitrosonium cation are related to nitric oxide by reduction and oxidation of $NO^\bullet$ respectively, and each has a distinctive chemistry unique to itself [3]. The fundamental chemistry of these three species is best rationalised in terms of MO theory. The nitroxyl anion, isoelectronic with dioxygen, has a bond order of 2. Nitric oxide is a radical, with a bond order of 2.5, the unpaired electron occupying an antibonding π orbital. Nitric oxide does not show a strong tendency to dimerise. Loss of the unpaired electron gives NO^+, which has a bond order of 3 and is isoelectronic with N_2 and CO. Nitric oxide is difficult to oxidise, with a reduction potential of 1.2 V. The electron affinity of $NO^\bullet$ will depend upon whether the NO^- product is in the singlet or triplet state, but the reduction of nitric oxide to the nitroxyl ion can be carried out by biological reducing agents. Nitroxyl ion is formed by reaction of $NO^\bullet$ with hydrated electrons, although under these conditions, the nitroxyl ion then reacts with NO to give $(NO)_2^-$ and $(NO)_3^-$ [4–6]. Nitric oxide is also reduced to NO^- by Fe(II) in acidic solution, while nitroxyl ion can be oxidised to NO by ferricyanide ion, $[Fe(CN)_6]^{3-}$.

Interconversion of NO^-, $NO^\bullet$ and NO^+ can take place under cellular conditions, so all three species must be considered in order to account fully for the biological activity of nitric oxide. The diversity and complexity of such activity is consistent with the view that various species derived from NO play important biological roles [7]. Indeed, NO^-, $NO^\bullet$ and NO^+ each exert unique toxic effects on *Clostridium sporogenes* [8].

2. The nitroxyl anion, NO^-

This review will focus on the nitroxyl ion as the importance of this species has been overlooked or rejected in the past. However, it must now be accepted that nitroxyl ion can be formed in the cellular milieu by several routes and that it can exert direct and varied effects on biological molecules and systems. Thus, it has been proposed that nitroxyl ion may carry out reactions typical of the endothelial-derived relaxing factor (EDRF), although the possibility that nitroxyl ion is oxidised to NO before these reactions occur cannot be excluded. Claims have been made that nitroxyl ion is formed directly by nitric oxide synthase (NOS). Furthermore, its chemistry and biology are linked to that of peroxynitrite as the nitroxyl ion reacts with dioxygen to give peroxynitrite (Eq. 2). This reaction occurs in the autoxidation of hydroxylamine in alkaline solution [9], one route for the preparation of peroxynitrite. Several reports have shown that co-ordination of NO to an Fe(II) haem protein leads to reduction to give an NO^- nitrosylhaem group which may then be followed by release of the nitroxyl ion. This may result

in the formation of peroxynitrite under aerobic environments and so this pathway must also be considered in any assessment of the likelihood and extent of intracellular formation of peroxynitrite. The parent acid of the nitroxyl ion (HNO) has $pK_a = 4.7$ and so is fully deprotonated at biological pH values

$$NO + O_2^- = ONOO^- \quad (1)$$

$$NO^- + O_2 = ONOO^- \quad (2)$$

Methods for the chemical generation of nitroxyl ion in solution have recently been described [10].

3. Biochemical pathways for the production of nitroxyl ion

It was shown some time ago by Murphy and Seiss that superoxide dismutase catalyses the reversible reduction of nitric oxide to nitroxyl ion [11]. The formation of nitroxyl ion can be confirmed by trapping with metmyoglobin (Fe(III)) [12].

As noted above, the binding of nitric oxide to iron(II) in a haem centre may lead to the release of nitroxyl anion, which in the absence of other reactions will dimerise to give nitrous oxide. Examples include the nitric oxide reductase of *Paracoccus denitrificans* [13], cytochrome *c* [14] and haemoglobin [15]. In the case of cytochrome *c*, the released nitroxyl ion reacted with dioxygen to give peroxynitrite, shown by the oxidation of dihydrorhodamine 123 [14]. In contrast to these examples of reductive formation of nitroxyl ion, the oxidation of azide ion by lignin peroxidase also leads to the release of nitroxyl ion from the iron centre in the enzyme [16].

Nitroxyl ion may also be formed by the oxidative decomposition of *N*-hydroxyarginine, the intermediate formed during the NOS-catalysed oxidation of arginine by oxygen [17]. There are also claims that NO^- is released under some conditions during the NOS-catalysed oxidation of L-arginine [18], but these results may need further consideration.

Arnelle and Stamler have shown that the decomposition of *S*-nitrosothiols in the presence of thiols leads to the formation of hydroxylamine and nitrous oxide, and have interpreted these results in terms of a reaction between nitroxyl ion (donated by the *S*-nitrosothiol) and thiols (Eqs. 3 and 4) [19]. The trapping of nitroxyl ion by thiols has been reported earlier by Doyle et al. [20] and Turk and Hollocher [13].

$$RSH + HNO \rightarrow RSNHOH \xrightarrow{RSH} RSSR + NH_2OH \quad (3)$$

$$2HNO \rightarrow N_2O + H_2O \quad (4)$$

This reaction is important as it extends the modes of reactivity of *S*-nitrosothiols. These compounds undergo homolytic cleavage to give $NO^\bullet$ (and are often used as $NO^\bullet$ releasers) although in practice they are usually more reactive as nitrosating agents (that is, they donate the NO^+ group to an nucleophile of appropriate reactivity). The possibility that *S*-nitrosothiols can also donate nitroxyl ion to thiols emphasises further the intracellular significance of the species formed by oxidation or reduction of $NO^\bullet$ and has consequences in terms of redox balance that may be significant in regulatory and other processes. Furthermore, the hydroxylamine-producing reaction leads to the formation of S–S bonds and so could simultaneously induce conformational changes in enzymes or receptor sites.

4. Reactions of nitroxyl ion

The nitroxyl ion is a short-lived species in solution, decomposing via dimerisation and dehydration to give nitrous oxide (Eq. 4). The second-order rate constant for the dimerisation of HNO is 1.8×10^9 $dm^3\ mol^{-1}\ s^{-1}$ [4]. The rate of dimerisation will vary considerably with concentration in view of the square dependence on $[NO^-]$ in the rate law. At neutral pH values, the life time of transient concentrations of nitroxyl ion is likely to be milliseconds.

Nitroxyl ion reacts with a variety of targets. Some of these reactions may be useful for establishing the presence of nitroxyl ion as a reaction intermediate. The reaction with thiols is a good example of such reactions and is discussed later. Alternatively, the nitroxyl ion dimerisation reaction may be exploited to show conclusively the formation of NO^- by allowing a reaction thought to produce NO^- to take place in the presence of ^{15}N sodium trioxodinitrate ($O^{15}N=NO_2^{\ 2-}$) which releases $^{15}NO^-$ on decomposition. If nitrous oxide with mass number 45 is

formed, then cross dimerisation must have occurred between the $^{15}NO^-$ released from the ^{15}N-labelled trioxodinitrate and $^{14}NO^-$ produced in the reaction under study. Unlabelled trioxodinitrate may be used if the NO^- source molecule is ^{15}N-labelled at the appropriate point. Another approach to testing for the intermediacy of the nitroxyl ion exploits the fact that the nitroxyl anion reacts rapidly with NO in a reaction that is close to diffusion controlled [4–6]. The addition of an NO releaser to a reaction should therefore inhibit any reaction attributable to nitroxyl anion. In these approaches, of course, complications may arise from the addition of the thiol or the NO^- and NO releasers.

4.1. Reaction with metal centres to give nitrosyl complexes

Nitroxyl ion formed during autoxidation of hydroxylamine at high pH reacts with oxygen to give the peroxynitrite anion [9]. The intermediacy of the nitroxyl ion in this process was confirmed by trapping with the anion $[Ni(CN)_4]^{2-}$ to give the violet tricyanonitrosylnickelate(II) anion, in which the nitrosyl group is present as NO^- [9]. Nitroxyl ion formed by decomposition of the hydrogentrioxodinitrate ion, $HN_2O_3^-$, has been trapped using methaemoglobin or metmyoglobin [12]. The nitrosyl product is EPR-detectable. Cytochrome *d* is nitrosylated by the nitroxyl ion released from trioxodinitrate [21]. The nitroxyl ion, released from trioxodinitrate, has a specific inhibitory effect on the production of hydrogen via the phosphoroclastic pathway in *C. sporogenes*: this has been attributed to the binding of the nitroxyl ion at an iron–sulfur centre [8].

4.2. Reaction with thiols

The reaction with cysteine (Eq. 3) has been used to establish the formation of nitroxyl ion. High concentrations of cysteine cause complete inhibition of the vasorelaxant activity of the trioxodinitrate group [22], confirming the involvement of nitroxyl ion, although this does not eliminate the possibility that the nitroxyl ion serves as a source of nitric oxide via oxidation. Sharpe and Cooper [14] have confirmed that ferrocytochrome *c* reduces nitric oxide to NO^- by using the yeast ferrocytochrome *c*, which contains a free cysteine group (unlike horse heart cytochrome *c*), and observing the formation of dimers cross-linked via the L-cysteine residues.

4.3. Reaction with the oxygen molecule

The reaction between nitroxyl ion and dioxygen to give peroxynitrite is of particular significance. Can this be a stoichiometrically significant route for the production of peroxynitrite compared to the pathway involving nitric oxide and superoxide? Second-order rate constants are 5.7×10^7 dm^3 mol^{-1} s^{-1} and 1.9×10^{10} dm^3 mol^{-1} s^{-1}, respectively [23], favouring the superoxide–nitric oxide pathway by a factor of 300. However, intracellular concentrations of superoxide are very low, due to the efficient catalysis of its disproportionation by superoxide dismutase. Basal release of superoxide from cultured endothelial cells is too low to be measured [24]. Concentrations of dioxygen could be up to a thousand times greater than those of superoxide, supporting the suggestion that the reaction between dioxygen and nitroxyl ion is a significant peroxynitrite source. However, assessment of the rate of reaction between nitroxyl ion and dioxygen is complicated by two factors. The concentration of the nitroxyl ion is difficult to estimate (there are competing reactions, for example with haem and iron–sulfur centres and dimerisation). The second factor involves the spin state of the nitroxyl ion, which, like the isoelectronic dioxygen, can exist in singlet or triplet states, with zero or two unpaired electrons respectively. However, while dioxygen has a triplet ground state, NO^- has a singlet ground state. Nitroxyl ion in the singlet state does not react with dioxygen. The thermal decomposition of trioxodinitrate (known to give singlet nitroxyl ion) in oxygenated aqueous solution in the pH range 7–8 and at concentrations around 7×10^{-5} mol dm^{-3} does not lead to the formation of nitrate, which should be present if peroxynitrite had been formed at these concentrations (M.N. Hughes, Bonner F.T. and M. Sherry, unpublished data). It appears, therefore, that dioxygen reacts with the excited, triplet state of the nitroxyl ion. Thus, the magnitude of the pathway for peroxynitrite formation involving nitroxyl ion is difficult to place on a quantitative basis, especially as the electronic state of the nitroxyl ion produced by particular pathways may not be

known. Nevertheless, Sharpe and Cooper [14] have produced evidence to show that the nitroxyl anion formed by reduction of $NO^{\bullet}$ by ferrocytochrome *c* reacts with dioxygen to form peroxynitrite. These results show that peroxynitrite can be formed in the absence of superoxide by the reaction between dioxygen and nitroxyl ion, a conclusion that emphasises the potential significance of peroxynitrite as a cytotoxic agent.

4.4. Cytotoxicity of nitroxyl ion

Nitroxyl ion may exert toxic effects apart from those arising from the formation of peroxynitrite. It inhibits hydrogen production via the phosphoroclastic pathway in *C. sporogenes* by binding to an iron–sulfur cluster, as shown by EPR spectroscopy [8]. Wink et al. [25] have shown that sodium trioxodinitrate is toxic to Chinese hamster V79 lung fibroblast cells at concentrations of 2–4 mM. The presence of ferricyanide in equimolar amounts protected against these effects by oxidising the nitroxyl ion to NO [25,26]. The toxicity of the trioxodinitrate was enhanced under aerobic conditions. Exposure of the cells to trioxodinitrate resulted in double strand breaks in DNA. Peroxynitrite does not induce this type of cellular damage and so these effects are assumed to be brought about by the nitroxyl anion.

5. Chemical routes for generating nitroxyl ion

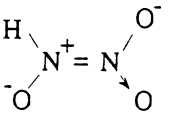
Nitroxyl ion is usually generated in solution by the decomposition of either Angeli's salt, $Na_2N_2O_3$ (via the hydrogentrioxodinitrate(1−) ion, $HN_2O_3^-$) or Piloty's acid, *N*-hydroxybenzenesulfonamide (which is commercially available). It should be noted that the salt 'NaNO', prepared by the reaction of nitric oxide with sodium in liquid ammonia, is dimeric and does not act as a source of NO^- in solution. The rate of reactions involving the nitroxyl ion will normally be determined by the rate of decomposition of the nitroxyl ion generating species. The concentrations of nitroxyl ion present in solution will be uncertain.

The synthesis of sodium trioxodinitrate, $Na_2N_2O_3{\cdot}H_2O$, is shown in Eq. 5 [27,28]. A variety of organic nitrates have been used, including ethyl, isopropyl and butyl nitrates. The anhydrous form may be obtained by drying at 100°C.

$$NH_2OH + RONO_2 + 2NaOC_2H_5 \rightarrow ROH + Na_2N_2O_3 + 2C_2H_5OH \quad (5)$$

The stoichiometry of the decomposition of trioxodinitrate is pH-dependent [29]. At pH values below 4, the only product is nitric oxide. This reaction is initiated by nitrite and its onset at around pH 4 probably reflects the protonation of nitrite to give nitrous acid, followed by nitrosation of the hydrogentrioxodinitrate anion $HN_2O_3^-$. At pH values above 4, the products are nitrous oxide (via dimerisation of nitroxyl ion) and nitrite. The formation of nitrite alongside nitroxyl ion means that control experiments must be carried out with nitrite to confirm that any effects observed on a biological process are not due to nitrite ion. If ^{15}N hydroxylamine is used to synthesise sodium trioxodinitrate(2−), then ^{15}N nitroxyl ion will be produced on decomposition.

The first order rate constant for the decomposition of trioxodinitrate is independent of pH between pH 4.4 and 8.1 and its value at 25°C is 6.75×10^{-4} s^{-1}, with a half life of about 17 min. At 37°C, these values are 4.1×10^{-3} s^{-1} and 2.8 min, respectively [29,30]. The pK_a values for $H_2N_2O_3$ and $HN_2O_3^-$ are 2.39 and 9.36, respectively. Thus the reacting species is $HN_2O_3^-$. Trioxodinitrate under these conditions of pH is stabilised by added nitrite, a product of the reaction, showing that an equilibrium exists between $HN_2O_3^-$ and the immediate products of nitrogen–nitrogen bond cleavage (Eq. 6) [31].



(6)

This reaction cannot involve cleavage of the nitrogen–nitrogen double bond in trioxodinitrate, as this would give products in the triplet electronic state. The reverse reaction involves nitrite ion in the ground state and therefore singlet nitroxyl ion. By the principle of microscopic reversibility, the forward reaction must therefore also involve singlet nitroxyl ion. Therefore, the decomposition of the hydrogentrioxodinitrate ion involves cleavage of an nitrogen–nitrogen single bond, to give singlet nitroxyl ion. The rate-determining step has accordingly been suggested

[31] to involve tautomerisation of the species with a nitrogen–nitrogen double bond into one with a nitrogen–nitrogen single bond, which then undergoes N–N bond cleavage.

Piloty's acid brings about vasodilation, which has been attributed to the release of the nitroxyl ion. However, the decomposition of this compound to release NO^- in aqueous solutions is extremely slow at neutral pH ($t_{1/2}$ about 80 h, at 25°C) and studies must be carried out under anaerobic conditions to prevent rapid oxidative decomposition of Piloty's acid [32]. It appears probable that the guanylyl cyclase-stimulating, vasodilator and anti-platelet activity of Piloty's acid are due to the nitric oxide released by this oxidative process [26]. The fact that cysteine has no effect on the biological activity of Piloty's acid under these conditions confirms that this is not due to the formation of nitroxyl ion [26].

Values of the first-order rate constant for the decomposition of Piloty's acid to nitroxyl ion increase with pH, as the anion of Piloty's acid is the kinetically active species. The pK_a of Piloty's acid is 9.29, so the values of k_1 level off by about pH 11, with a maximum value of $k_1 = 4.22 \times 10^{-4}$ s^{-1} when Piloty's acid is fully deprotonated (Eqs. 7–9). At pH values 7.40 and 8.00 at 25°C, the half lives for the decomposition of Piloty's acid are 35 and 6 h, respectively [30].

$$C_6H_5SO_2NHOH + OH^- = C_6H_5SO_2NHO^- + H_2O \quad (7)$$

$$C_6H_5SO_2NHO^- = HNO + C_6H_5SO_2^- \quad (8)$$

$$HNO = H^+ + NO^- \quad (9)$$

Addition of the product benzenesulfinate anion results in decreased rates of decomposition of Piloty's acid, suggesting reversibility of the decomposition of the anion (Eq. 8). This confirms that the other decomposition product must be nitroxyl ion and suggests that the singlet state anion is released from Piloty's acid, as well as from trioxodinitrate. Some inhibitory effects of Piloty's acid on the growth of food spoilage and other bacteria under anaerobic conditions in media around pH 8 have been attributed to the release of the nitroxyl ion (Hughes, Cammack, Khan and Torres Martinez, unpublished work).

6. Photochemical decomposition of trioxodinitrate

As noted above, self-decomposition of trioxodinitrate to give nitrite and nitroxyl ion in the presence of dioxygen does not lead to the formation of peroxynitrite. In contrast, the photochemical decomposition (using the mercury line at 253.7 nm) of the trioxodinitrate anion in the presence of dioxygen does give peroxynitrite [33], as shown by the formation and subsequent decay of a species with maximum absorbance at 300 nm. Peroxynitrite was not seen at pH 8, in accordance with its known instability and probably the photolysis of peroxynitrous acid itself. The formation of peroxynitrite was observed at higher pH values. In 0.1 mol dm^{-3} NaOH solution, the conversion of trioxodinitrate to peroxynitrite was stoichiometric. Nitrite was also formed. Peroxynitrite was not formed when the photochemical decomposition was carried out using dinitrogen-saturated solutions, the products being nitrite (on a 1:1 stoichiometry) and nitrous oxide. This suggests that the triplet state nitroxyl ion formed in the photochemical decomposition of trioxodinitrate is converted quantitatively to peroxynitrite.

7. Electronic states of the nitroxyl ion

The nitroxyl ion is isoelectronic with the oxygen molecule, which exists in a triplet ground state with two unpaired electrons in the degenerate π antibonding orbitals. In the singlet excited state, the electrons are paired to give a diamagnetic molecule. In the gas phase, NO^- has a triplet ground state, while the singlet state is 72 kJ mol^{-1} higher in energy [34]. However, as argued above, the nitroxyl ion formed on thermal decomposition in aqueous solution of the hydrogentrioxodinitrate anion is in the singlet, ground electronic state, and does not react with molecular oxygen. The nitroxyl anion formed on photochemical decomposition in aqueous solution of trioxodinitrate anion is in the triplet state and reacts with molecular oxygen to give peroxynitrite. The nitroxyl ion is a heteronuclear species with the negative charge localised on the nitrogen atom (the parent acid is HNO). This must lead to sufficient splitting of the degeneracy of the π antibonding orbitals to result in a singlet ground state for the nitroxyl ion.

Stanbury [35] has calculated the reduction potentials for the NO/$^1NO^-$ and NO/$^3NO^-$ couples to be −0.35 and 0.39 V, respectively. The latter value is consistent with the suggestion that NO$^\bullet$ can be reduced to NO^- by the hexaammineruthenium(II) cation [36].

8. The nitrosonium cation, NO^+

This cation, the oxidised form of nitric oxide, is related to nitrous acid and is the key species in the process of nitrosation, in which the NO^+ group is transferred (usually from a carrier compound) to a nucleophilic centre, often to a sulfur or nitrogen lone pair of electrons [37,38]. The nitrosonium cation is rapidly hydrolysed in aqueous solution to give nitrous acid (Eq. 10). The equilibrium constant for this reaction is $10^{-6.5}$ at 25°C [38] and the life time of NO^+ in water is about 3×10^{-10} s.

$$NO^+ + H_2O = H^+ + HNO_2 \quad (10)$$

The use of aqueous solutions of salts containing the nitrosonium cation (e.g. $[NO^+][BF_4]$ or $[NO^+][PF_6^-]$) in the expectation that these solutions contain the nitrosonium cation is unfortunate. The NO^+ cation is only found in aqueous solution at very high acidity. At pH values of biological relevance, nitrosation is most likely to occur through the action of NO^+ carriers such as *S*-nitrosothiols or possibly *N*-nitrosamines. *S*-Nitrosoglutathione is an excellent nitrosating agent, for example, and can transfer the NO^+ group to a variety of nucleophiles in the process now commonly called transnitrosation [37]. Nitrosyl complexes in which the nitrosyl group is formally NO^+, for example sodium nitroprusside, $Na_2[Fe(CN)_5(NO^+)]$, may be very effective nitrosating agents at pH 7, particularly towards sulfur centres. Such NO^+ nitrosyl complexes may be formed intracellularly by reaction of NO with Fe(III) centres.

Nitrosation at pH values of 7 and above by NO and NO_2 have been studied by Challis and his coworkers [39]. Nitric oxide cannot in isolation act as a nitrosating agent. It will function in this capacity in the presence of dioxygen [40] or other oxidising agents, such as metal salts, because then NO will be oxidised to a species containing NO^+. Alternatively, a radical substrate species might be formed that will react with NO.

Nitrosation is an important process in cell biochemistry. Receptor sites in control and signalling pathways often contain thiol groups. Nitrosation of these thiol groups (RS^-) to give *S*-nitrosothiols (RSNO) could lead to homolysis of the S–N bond with loss of nitric oxide, the formation of S–S bonds and conformational changes with considerable effects upon cells. It is also possible that a series of transnitrosations could occur, with the NO^+ group being transferred from one carrier to another, until a critical nitrosation step takes place. Compounds that act as NO^+ donors are effective triggers of apoptosis in Swiss 3T3 fibroblasts [41] and neuronal PC12 cells [42].

9. Peroxynitrite, $ONOO^-$

There is an extensive literature on peroxynitrite. Much of this deals with reactions of peroxynitrite with biological targets, that is covered elsewhere in this issue. These reactions may involve nitration and/or hydroxylation, as in the nitration of tyrosine residues in proteins. Peroxynitrous acid is a stronger oxidising agent than either nitric oxide or superoxide, from which it may be formed, and readily oxidises thiols, ascorbate and lipids. It can carry out one-electron oxidations and two electron oxidations (by oxygen atom transfer).

It is generally accepted that activated macrophages produce peroxynitrite via the formation of NO and reaction with O_2^- [43]. While peroxynitrite production by macrophages is beneficial, its formation elsewhere is harmful [23] causing oxidative damage to biological tissues and giving rise to various pathogenic conditions. The toxic effects of peroxynitrite to cells and mitochondria is, therefore, a topic of interest [44]. Its short life time of about 1 s at physiological pH values presents difficulties in such studies, although this life time is sufficient to allow peroxynitrite to diffuse to crucial targets. As noted earlier, there is evidence to show that the alternative route for peroxynitrite formation, involving the reaction between dioxygen and nitroxyl ion, does occur [14].

Peroxynitrite reacts rapidly with carbon dioxide to

give an adduct of composition $ONO_2CO_2^-$ [45–48]. Carbonate concentrations in physiological fluids are about 25 mmol dm^{-3}, so the formation of this adduct takes may place more rapidly than do other reactions associated with peroxynitrite toxicity. If this is the case, then, depending on the toxicity of this adduct, carbonate may either enhance peroxynitrite toxicity or protect against it.

Peroxynitrous acid ONOOH may be regarded as a carrier of NO^+, although there is only limited evidence to suggest that it can react as a nitrosating agent. The presence of this group has a major influence on the peroxynitrous acid molecule. The strong inductive effect of NO^+ causes a weakening of the O–O bond compared to that in hydrogen peroxide, with bond strengths of 90 and 170 kJ mol^{-1}, respectively. It also results in peroxynitrous acid having one of the lowest pK_a values of any peroxo acid, with peroxynitrous acid and hydrogen peroxide having pK_a values of about 6.8 (see [49]) and 15.7, respectively. Peroxynitrous acid isomerises to nitrate at physiological pH values, although nitrite may also formed extensively under some conditions, for example, in the presence of trace metals. The peroxynitrite anion is stable with respect to decomposition. The question of whether the decomposition and reactions of peroxynitrous acid involves homolysis of the O–O bond to give OH and NO_2 radicals, which either may recombine to give nitrate or react with substrates, has been the subject of debate for many years. Recently, stopped flow studies on the isomerisation of peroxynitrous acid at high pressure have shown that the rate of isomerisation depends little on the pressure, with a volume of activation of 1.7 cm^3 mol^{-1} [49]. This is not consistent with a mechanism of isomerisation in which the rate-determining step is homolysis of the peroxide bond to give free hydroxyl and nitrogen dioxide radicals. However, ^{15}N CIDNP NMR studies on the nitration of tyrosine suggest that this reaction occurs by a radical process, implying that peroxynitrite does cleave homolytically [50]. Nevertheless, it has been pointed out that the question of whether or not peroxynitrite undergoes homolysis or reacts via a *trans* intermediate is not relevant to biology. Peroxynitrite formed in blood vessels will react with carbon dioxide while peroxynitrite formed in cells will oxidise thiols [51].

10. Conclusions

This survey of the reactions of the three simple diatomic species NO^-, $NO^\bullet$ and NO^+ and the relationships between them has demonstrated the complexity of their chemistry and the remarkable diversity of their biological behaviour. All this reflects the non-innocence of the nitric oxide molecule. This flexibility in chemical behaviour is maintained in intracellular molecules or ions such as *S*-nitrosothiols and iron nitrosyl complexes, which maintain the redox flexibility of the NO group, effectively serve as a store of these species and allow essential features of their chemistry to be manifested at pH 7. The rich chemistry of small nitrogen compounds continues to be demonstrated in the reactions of the peroxynitrite group, for which a number of issues are still open to debate.

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