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Peroxynitrite: an endogenous oxidizing and nitrating agent

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Abstract. Peroxynitrite, the reaction product between nitric oxide ($^{\bullet}NO$) and superoxide, has been presumed to be a mediator of cellular and tissue injury in various pathological situations. It is formed at the convergence of two independent radical-generating metabolic pathways. Its biological effects are due to its reactivity towards a large range of molecules including amino acids such as cysteine, methionine, tyrosine and tryptophan, nucleic bases and antioxidants (e.g. phenolics, selenium- and metal-containing compounds, ascorbate and urate). Peroxynitrite reactions involve oxidation and nitration. The chemical properties depend on the presence of CO_2 and metallic compounds as well as the concentrations of reagents and kinetic laws. This complex chemistry can be explained by the formation of several structural forms and active intermediates released from peroxynitrite.

Key words. Peroxynitrite; oxoperoxonitrate; oxidative stress; nitric oxide; superoxide.

Nitric oxide ($^{\bullet}NO$) has been thought to be the primary mediator of cellular and tissue injury under pathological conditions. However, recent studies have suggested that $^{\bullet}NO$ might play a cytoprotective role by acting as an antioxidative agent [1–3] or as a free radical chain terminator [4]. Peroxynitrite[1], a reaction product between $^{\bullet}NO$ and superoxide, has been considered as an oxidant and nitrating mediator formed in various pathological situations [5–7]. Here we will review reactions of peroxynitrite with biologically relevant molecules.

Chemical synthesis and biosynthesis

Several methods for the laboratory synthesis of peroxynitrite have been reported, including photolysis and pulse radiolysis of nitrate [8], ozonation of azide [9], oxidation of hydroxylamine [10], oxidation of organic nitrate [11] and the reaction of $^{\bullet}NO$ with tetramethyl ammonium superoxide or potassium superoxide [12]. Optimal conditions for the generation of peroxynitrite by the quenched flow reaction of nitrite with hydrogen peroxide under acidic conditions have recently been reported [13].

Peroxynitrite formation in vivo is a diffusion-controlled reaction of $^{\bullet}NO$ and superoxide radicals [6, 14]. In biological systems, autoxidation of $^{\bullet}NO$ may not be responsible for the short half-life of $^{\bullet}NO$ (3–5 s) measured under normal and pathological conditions [15]. However, the short half-life of $^{\bullet}NO$ may be explained, at least in part, by a rapid reaction between $^{\bullet}NO$ and the superoxide anion, which yields peroxynitrite ($4–20 \times 10^9\ M^{-1}\ s^{-1}$) [12].

The reaction of the two radicals, $^{\bullet}NO$ and superoxide, is 30 times faster than that of $^{\bullet}NO$ with oxyhaemoglobin and 3 times faster than the dismutation of superoxide catalysed by superoxide dismutase (SOD) [16]. There are a variety of pathophysiological conditions in which production of both $^{\bullet}NO$ and $O_2^{\bullet -}$ is significantly elevated. However, formation of peroxynitrite depends on a delicate balance between the production of $O_2^{\bullet -}$ and SOD and $^{\bullet}NO$ synthesis and consumption [17].

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[1] The IUPAC-recommended name for O=N-O-O− is oxoperoxonitrate, for its conjugated acid, it is hydrogen oxoperoxonitrate, and that for $^{\bullet}NO$ is nitrogen monoxide. Nevertheless, peroxynitrite is commonly used for oxoperoxonitrate or the mixture with its conjugated acid form.

The presence of SOD preserves $^{\bullet}NO$ generated from L-arginine by $^{\bullet}NO$ synthases, suggesting that $^{\bullet}NO$ reacts rapidly with superoxide [18]. It has been reported that all three isoforms (neuronal, inducible and endothelial) of $^{\bullet}NO$ synthases produce both $^{\bullet}NO$ and $O_2^{\bullet -}$ simultaneously under certain conditions, especially, when the substrate L-arginine is deficient. Thus peroxynitrite is probably formed by $^{\bullet}NO$ synthases [19–22]. However, since direct measurements of peroxynitrite in vivo cannot be easily achieved, there is no direct evidence showing that peroxynitrite is formed in vivo. Oxidation of dihydrorhodamine has been used to measure the oxidizing activity of peroxynitrite formed from simultaneous fluxes of superoxide and $^{\bullet}NO$ but is significant only in the absence of low-molecular-weight iron [23]. The presence of nitrotyrosine has been measured routinely as a marker for peroxynitrite formation in vivo. However, recent studies have demonstrated that a variety of nitrating agents can react with tyrosine to form nitrotyrosine in vitro and in vivo [24].

Life span

The peroxynitrite anion ($ONOO^-$) is relatively stable but its acid form (ONOOH) decays to nitrate with a half-life of at most 1 s at physiological pH and temperature. Thus, peroxynitrite could be considered as a relatively long lived species. Only the *trans* isomer is observed by Raman spectroscopy, but calculations predict that the *cis* isomer is more stable than the *trans* isomer. Stabilization of the *cis* form by delocalization of the negative charge over the entire molecule explains the particularly low pK_a of 6.8 of the peroxide. Only the *trans* isomer could rearrange to nitrate by attacking the nitrogen [25].

Recently, Kissner et al. [12] reported that the pK_a of peroxynitrous acid (ONOOH) depends on buffer composition and concentration. It can vary from 6 to more than 8. The thermodynamically favourable modes of peroxynitrite decay are isomerization to nitrate and dismutation either to nitrogen dioxide and nitrosodioxyl radicals ($ONOO^{\bullet}$) or to nitrite and oxygen:

$$ONOOH \rightarrow HNO$$

$$2\ ONOOH \rightarrow H_2O + ONOO^{\bullet} + {}^{\bullet}NO_2$$

$$2\ ONOO^- \rightarrow O_2 + 2\ NO_2^-$$

The kinetics of peroxynitrite decay are strictly a first-order reaction at a rate of 1.3 s^{-1} at 25 °C [26]. Different pathways of decomposition account for the formation of nitrate or nitrite and O_2. Pfeiffer et al. [27] reported that nitrite (30%) and oxygen (15%) are formed from peroxynitrite in aqueous solution at pH 7.5, whereas NO_3^- is the sole product at $pH < 5$. In most buffers, an acceleration of the decay is observed with increasing buffer concentration [28]. Peroxynitrite is less stable in the presence of bicarbonate and carbon dioxide, which behave as a Lewis acid [29]. When the concentration of peroxynitrite exceeds 100 μM, its disappearance seems to be delayed as peroxynitrite and peroxynitrous acid are stabilized by forming a pair which undergoes a slower decay leading also to oxygen and nitrite [12].

$$HOONO + ONOO^- \rightarrow [ONOO/ONOOH]^- \rightarrow O_2 + NO_2^-$$

Although peroxynitrite shows a half-life of around 1 s at physiological pH and temperature, the exact life span of peroxynitrite in vivo is not known. $^{\bullet}NO$ diffuses freely across membranes, whereas $O_2^{\bullet -}$ and the peroxynitrite anion need anion channels to cross membranes. In fact, recent studies with whole red blood cells showed clearly that peroxynitrous acid can diffuse across the lipid bilayer, while transmembrane transport of peroxynitrite anion occurs through the erythrocytic anion channel. In the presence of extracellular targets like CO_2, peroxynitrite diffusion through membranes will be limited, suggesting its decay in the same compartment [30].

Aromatic hydroxylation and oxidation by peroxynitrite

Hydroxylation by peroxynitrite of phenylalanine at the three positions of the aromatic ring [31] and of salycilic acid at *ortho* and *para* positions [32] have been observed in vitro, although their yields were low. Similar amounts of hydroxylated products are obtained with hydroxyl radical ($HO^{\bullet}$)-generating systems (iron-EDTA, ascorbate and hydrogen peroxide) [32]. This hydroxylation reaction by peroxynitrite could be explained by $HO^{\bullet}$ formation if homolysis of peroxynitrous acid took place. However, on the basis of thermodynamic calculations for homolysis ($10^{-2}\ s^{-1}$) and isomerization rate ($1\ s^{-1}$), homolysis seems unlikely to occur [33]. This is confirmed by the independence of the rate of decomposition from solvent viscosity [34]. However, formation of an activated intermediate of peroxynitrite, which can hydroxylate the benzene ring and react with $HO^{\bullet}$ scavengers such as deoxyribose and histidine, has been reported to be the vibrationally excited *trans* isomer of peroxynitrous acid [26, 35–37]. Oxidation yields with increasing concentra-

tions of substrates do not exceed 40%, suggesting that 40% of peroxynitrite is converted into this activated oxidizing species [38].[2]

Reaction with carbon dioxide/bicarbonate and carbonyls

Peroxynitrite anion reacts with CO_2 with a rate constant of 5.8×10^4 M^{-1} s^{-1} [39, 40]. The conformation of peroxynitrite may be an important determinant of its reactivity. The *trans* rotamer should be the reactant. Using carbonate anhydrase that catalyses the reversible hydration of CO_2 as a probe, CO_2 has been shown to be the reactive species [41]. As the concentrations of CO_2 (1.3 mM in blood plasma) and bicarbonate (12 mM in intracellular fluid and 25–30 mM in blood plasma) are high, this reaction between the peroxynitrite anion and CO_2 should occur in vivo and accelerate its decay [41]. Thus, biological activities of peroxynitrite are expected to occur via the intermediate nitrosodioxycarboxylate ($O=NOOCO_2^-$). The reaction of peroxynitrite with CO_2 enhances nitration reactions, whereas it inhibits hydroxylation and oxidation reactions mediated by peroxynitrous acid (ONOOH) [40, 42, 43] (fig. 1).

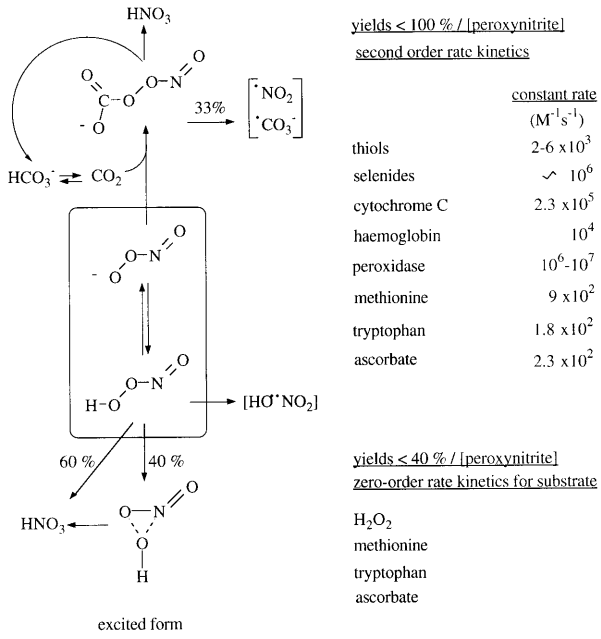

Figure 1. Reactions of oxidation by peroxynitrite.

It has been suggested that this peroxynitrite anion-CO_2 adduct can lead to reactive intermediates such as the nitronium cation, $^{\bullet}NO_2$ and $^{\bullet}CO_3$ radicals or nitrooxocarboxylate anion ($O_2N\text{-}O\text{-}CO_2^-$) by isomerization [44]. All these species are oxidizing and nitrating agents. The strong oxidizing $^{\bullet}CO_3$ radical should lead to superoxide formation via radical intermediates. Superoxide and the $^{\bullet}NO_2$ radical should react to give peroxynitrate ($O_2N\text{-}O\text{-}O^-$) which decomposes rapidly into nitrite and oxygen [44].

The reaction of peroxynitrite with pyruvate (apparent second-order rate $k = 88$ M^{-1} s^{-1} at pH 7.4) or with α-ketoglutarate leads to the carbon dioxide radical anion which reacts very rapidly with oxygen. Radical formation and oxygen consumption are inhibited by the addition of 10 mM bicarbonate [45].

Similarly, aldehydes react with peroxynitrite, but with apparent rates at least 40–300 times lower than the rate constant for the CO_2-peroxynitrite reaction [46]. However, the presence of some short-chain aldehydes, which can form adducts similar to the peroxynitrite-CO_2 adduct, has been shown to inhibit peroxynitrite-mediated nitration of phenolic compounds. The behaviour of this adduct excludes the hypothesis of NO_2^+ formation from the peroxynitrite-CO_2 adduct. Thus a radical mechanism is probably responsible for the nitration of phenolic compounds [46].

Oxidation, nitration and nitrosation of thiols by peroxynitrite

The main reaction of peroxynitrite with thiols is a two-electron oxidation leading to disulphides, this being the major mechanism in the peroxynitrite-mediated inactivation of various enzymes. Cysteines are certainly the most sensitive targets in proteins. The apparent second-order rate constants during the reaction of peroxynitrite with thiols, such as cysteine, glutathione and the single thiol group of albumin, are reported to be 1.3, 1.5 and 4.5×10^3 M^{-1} s^{-1}, respectively, at physiological pH and temperature [47, 48]. Peroxynitrite oxidizes sulphhydryls about 10^3 times faster than does H_2O_2 under the same conditions. At lower concentrations of thiols (thiol/peroxynitrite < 1.2) and at acidic pH, a one-electron oxidation process occurs, at least partially, through formation of thiyl radicals which were detected by direct electron-spin resonance (ESR) and spin-trapping studies. The peroxynitrite-dependent formation of such free radicals is revealed in blood plasma, in addi-

[2] Three recent publications suggest that peroxynitrous acid (ONOOH) homolytically decomposes to generate $HO^{\bullet}$ and $^{\bullet}NO_2$.
1. Merenyi G., Lind J., Golstein S. and Czalski G. (1998) Peroxynitrous acid homolyzes into $HO^{\bullet}$ and $^{\bullet}NO_2$ radicals. Chem. Res. Toxicol. **11:** 712-713.
2. Coddington J. W., Hurst J. K. and Lymar S. V. (1999) Hydroxyl radical formation during peroxynitrous acid decomposition. J. Am. Chem. Soc. **121**: 2438–2443.
3. Richeson C. E., Mulder P., Bowry V. W. and Ingold K. U. (1998) The complex chemistry of peroxynitrite decomposition: new insights. J. Am. Chem. Soc. **120**: 7211–7219.

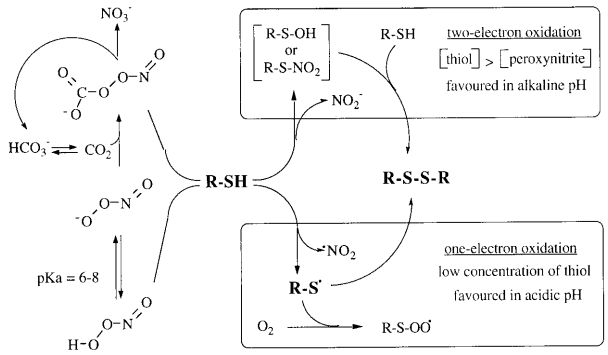

Figure 2. Mechanisms of thiol oxidation by peroxynitrite.

tion to ascorbyl, urate-derived and tryptophan-centred radicals [49–51]. Thiyl radicals can link oxygen, promoting oxidative stress. Electron paramagnetic resonance (EPR) and oxygen consumption experiments have led to a proposed mechanism shown in figure 2 [48].

Thiols and CO_2 could compete for peroxynitrite reactions. Rate constants for the reaction of peroxynitrite with carbon dioxide and with sulphhydryls have been reported as 5.8×10^4 M^{-1} s^{-1} and $2–5 \times 10^3$ M^{-1} s^{-1}, respectively. Considering these reaction rates and their respective concentrations in extra- and intracellular fluids, it may be possible that excited intermediate could not be involved in vivo. However, the participation of several oxidizing intermediates is supported by competition studies. The first-order rate for thiol concentration indicates that the ground-state form of peroxynitrous acid (ONOOH) can directly oxidize thiols. The presence of bicarbonate partially inhibits peroxynitrite-dependent oxidation of glutathione, indicating that the peroxynitrite-CO_2 adduct is less efficient in oxidation than peroxynitrous acid [40, 52]. Similar results have also been obtained with bovine serum albumin in vitro [40]. In total human blood plasma, the formation of thiyl radicals and disulphide cross-linking of the dimer of serum albumin (50% by 500 μM peroxynitrite) is greatly dependent on dissolved CO_2 and ascorbate [53]. Moreover, peroxynitrite (1–5 mM) also produces 5–14 μM thionitrite (albumin-S-NO) and thionitrate (albumin-S-NO_2), the yields of which double in the presence of CO_2 [53]. Such S-nitrosation/nitration of endogenous glutathione or protein thiols could explain some of the •NO-like activities observed with peroxynitrite [54].

Reactions of peroxynitrite with critical thiols are responsible for the inhibition of creatine kinase (with a rate constant of $8.8–10^5$ M^{-1} s^{-1}) [55], α-1-antiproteinase [56] and succinate dehydrogenase and fumarate reductase, which are critical enzymes for the energy metabolism in *Trypanosoma cruzi* [57]. •NO synthases are inhibited by peroxynitrite by irreversible oxidation of the haem thiolate bond located in the catalytic site [58].

Peroxynitrite can modify methionine via one-electron and two-electron processes. The one-electron pathway occurs only to a lesser extent under acidic conditions and with low substrate concentration, leading to a methionine sulphide radical cation, which decomposes to methional and ethylene [59]. The major reaction forms the two-electron oxidation product methionine sulphoxide with a rate constant of 280 M^{-1} s^{-1} at pH 7.4 [60]. As with thiols, added bicarbonate inhibits this oxidation [61]. Such modification of methionine by peroxynitrite has been reported to be responsible for the inactivation of several proteins and enzymes [62], such as α-1-antiproteinase [63], calmodulin [64] and *Escherichia coli* glutamine synthetase [61].

Nitration and oxidation of tyrosine by peroxynitrite

Both nitration and dimerization of tyrosine are two-electron processes. The reaction stoichiometry shows that at most 16 and 38% of the peroxynitrite are available for oxidation and nitration in the absence and presence of bicarbonate, respectively [65]. With regard to the degradation kinetics of peroxynitrite in the presence of CO_2, recent data [44] show that 33% of nitrosodioxycarboxylate ($O=N\text{-}O\text{-}O\text{-}CO_2^-$) is converted to reactants which could be the pair of radicals (eq. 1) or nitrooxocarboxylate ($O_2N\text{-}O\text{-}CO_2^-$) (eq. 2).

$$O=N\text{-}O\text{-}O^- + CO_2 \rightarrow O=N\text{-}O\text{-}O\text{-}CO_2^- \rightarrow O=N\text{-}O^\bullet + {}^\bullet O\text{-}CO_2^- \quad (1)$$

$$O=N\text{-}O\text{-}O\text{-}CO_2^- \rightarrow O_2N\text{-}O\text{-}CO_2^- \quad (2)$$

At present, the nitronium NO_2^+ is always excluded as the nitrating agent due to its very short life span, and from thermodynamic calculations. With regard to a radical mechanism, the formation of tyrosyl radical is a pH-dependent reaction that could be due to $^\bullet NO_2$ $^\bullet CO_3^-$ for example, with $^\bullet NO_2$, the rate constants are 3.2×10^5 M^{-1} s^{-1} at pH 7.5 and 7.5×10^6 M^{-1} s^{-1} at pH 9.6. Radical processes (via $O=N\text{-}O^\bullet + {}^\bullet O\text{-}CO_2^-$) account well for the identical $t_{1/2}$ for peroxynitrite disappearance (3 ms) and for tyrosine nitration (2.5 ms) [65]. On the other hand, the caged ion and radical pairs formed from the nitrosodioxycarboxylate may not diffuse out of solvent cages and thus cannot account for the formation of nitrite and nitrate ions detected in phosphate buffers with or without added bicarbonate. However, nitrooxocarboxylate anion ($O_2N\text{-}O\text{-}CO_2^-$) resulting from a rearrangement in the caged radical pair could also be the nitrating agent [41].

Effects of iron and metal-containing compounds

Transition metals including Fe^{3+}-EDTA and Cu/Zn-SOD can catalyse peroxynitrite-mediated nitration of phenolic compounds [66]. The nitronium-ion-like species derived from peroxynitrite could be responsible for the nitration:

$$ONOO^- + Fe^{3+}EDTA \rightarrow NO_2^+\text{--}O\text{--}Fe^{3+}EDTA$$

The nitronium part could effect an electrophilic substitution of the aromatic ring while the ferryl oxy group traps the released protons to yield water and the original catalyst. Furthermore, Fe^{3+}-EDTA promotes the nitration of tryptophan [67] and serotonin [68]. Cu-SOD does not exert any effect on nitration of indole derivatives but catalyses the nitration of specific tyrosine residues in some particular proteins such as neurofilament and, to a lesser extent, serum albumin [69]. Nitration of 4-hydroxyphenylacetic acid by peroxynitrite is catalysed by other metalloproteins such as catalase [70] and metal-containing substances such as ternary copper complexes and manganese (III) tetrakis(4-benzoic acid) porphyrin [71]. The presence of CO_2 inhibits metal-catalysed nitration by both Fe^{3+}-EDTA and Cu-SOD, which agrees with the preferential formation of the nitrosodioxycarboxylate over the other nitrating species [40].

Modifications of tyrosine-residues in proteins by peroxynitrite

Nitration of tyrosine (fig. 3) and tyrosine-containing proteins and their roles in pathophysiology have recently been reviewed by Ischiropoulos [24]. Bovine serum albumin contains 19 tyrosine residues: following its reaction with 1:1, 1:5 and 1:10 ratios of protein to peroxynitrite, 0.1, 1 and 3% of the tyrosine residues are nitrated and 0.02, 0.09 and 0.1% are oxidized to dityrosine, respectively [72]. Despite low yields of tyrosine modifications, nitration of tyrosine residues inactivates important proteins such as Mn-SOD [73, 74]. It has

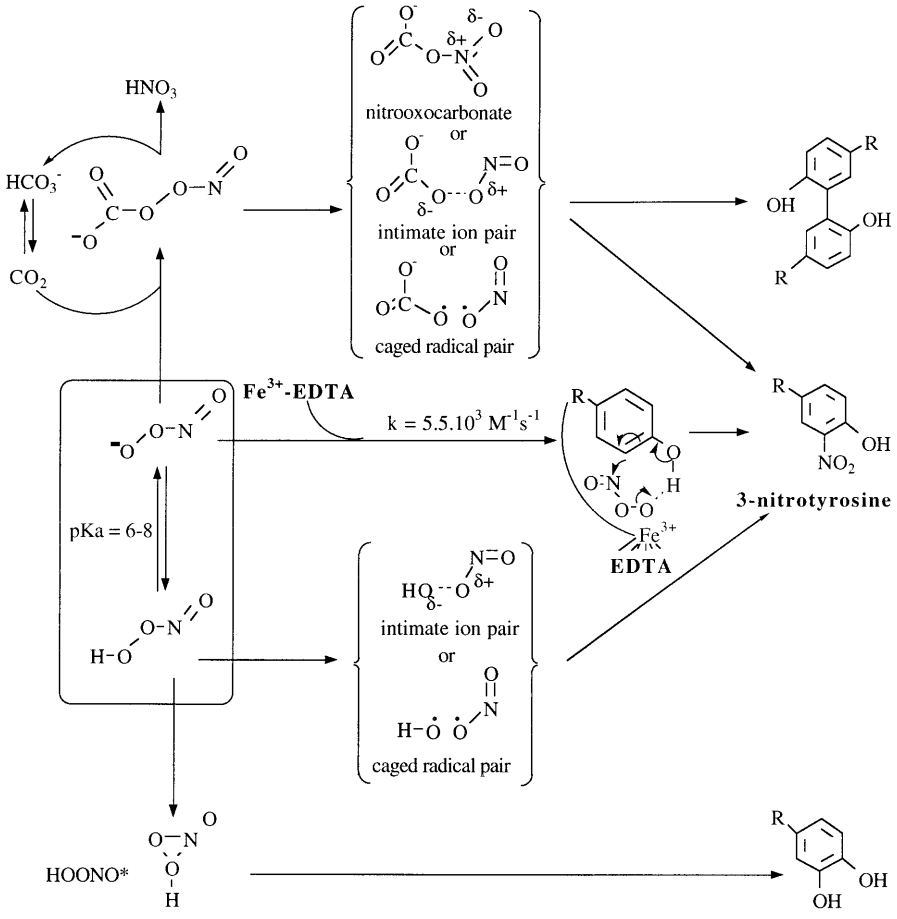

Figure 3. Mechanisms of tyrosine oxidation and nitration by peroxynitrite.

recently been reported that among the nine total tyrosine residues, only tyrosine 34 of Mn-SOD, which is located near the manganese and $O_2^{\bullet -}$ gateway, is nitrated by peroxynitrite [74]. Mn-SOD is nitrated during human kidney allograph rejection, strongly suggesting that peroxynitrite could be formed in vivo under certain pathophysiological conditions [75].

Other proteins, in which tyrosine nitration by peroxynitrite is associated with inactivation of protein functions/enzymatic activities, include neurofilament-L [69], prostacycline synthase [76], surfactant protein A [77], glutamine synthetase [61], tyrosine hydroxylase [78] and P450 [79]. Neurofilament-L, which is the major neurofilament subunit, maintaining axonal structural integrity, appears to be one of the predominant proteins in brain homogenates nitrated by peroxynitrite [69]. Nitrated neurofilament-L inhibits the assembly of unmodified neurofilament subunits. Regulation of glutamine synthetase activity of *E. coli* involves adenylation of tyrosine residues. Adenylation and nitration of critical tyrosine residues provoke changes in the affinity for substrates and allosteric effectors [61].

Nitration of tyrosine residues by peroxynitrite disrupts the phosphorylation of tyrosine residues in proteins involved in cell signalling networks [80]. However, recently, an enzymatic activity, which modifies nitrotyrosine-containing proteins (a 'nitrotyrosine denitrase'), has been reported in homogenates of rat tissue. The activity is increased about twofold in spleen extracts after endotoxin (bacterial lipopolysaccharide) treatment of animals, suggesting that the activity is inducible or regulated [81]. Thus, nitration of tyrosine could be one of the important post-translational modifications of proteins, the levels of which should be regulated biologically.

It should be noted that nitrotyrosine can be formed by various nitrating agents, including peroxynitrite, nitrous acid, N_2O_3 and NO_2 [24, 82, 83]. $^{\bullet}NO$ may also react directly with tyrosyl radicals (e.g. stable radicals such as that found in ribonucleotide reductase or those formed by H_2O_2 or others oxidants) to form nitroso or nitro derivatives. Prostaglandin H synthase-2, which exhibits a tyrosyl radical during the catalytic production of prostaglandins, has been reported to react with $^{\bullet}NO$ to form an iminoxyl radical which is in turn oxidized into nitrotyrosine [84]. Activated human polymorphonuclear neutrophils convert NO_2^- into NO_2Cl and the $^{\bullet}NO_2$ radical through myeloperoxidase-dependent pathways [85]. Myeloperoxidase and horseradish peroxidase utilize nitrite and hydrogen peroxide as substrates to catalyse tyrosine nitration in proteins [86]. Thus, the occurrence of nitrotyrosine-containing proteins in vivo should be regarded as a general indication of tissue damage induced by reactive nitrogen species such as peroxynitrite, NOx and HOCl plus nitrite in the presence of peroxidase.

Reactivity with tryptophan

Tryptophan can be nitrated by peroxynitrite on its benzene ring with an apparent second-order rate constant of 184 $M^{-1}\,s^{-1}$ at 37 °C. Nitration is moderately increased by Fe^{3+}-EDTA or by the addition of bicarbonate. Besides nitro derivatives, several oxidation products are formed. Peroxynitrite-mediated one-electron oxidation of tryptophan, leading to the tryptophanyl radical, is enhanced by bicarbonate [67]. The reaction of proteins with peroxynitrite has been reported to result in a decrease in tryptophan fluorescence, although the nature of the transformation is not specified. For instance, 12, 30 and 45% oxidation of tryptophan residues is observed following reaction of bovine serum albumin with peroxynitrite at ratios of 1:1, 1:5 and 1:10, respectively [72].

Reactivity with antioxidants

Phenolic compounds

Catecholamines, which act as antioxidants by donating electrons, react with peroxynitrite to form semiquinones and quinones [87]. The ability of different hydroxycinnamates to inhibit peroxynitrite-mediated tyrosine nitration has been compared to that of trolox. Ferulic and *p*- and *o*-coumaric acids undergo nitration by peroxynitrite, although catechol derivatives lead to relatively unstable products presenting the characteristics of quinones [88]. Furthermore, the ability of chlorogenic acid to protect DNA against peroxynitrite-induced oxidative damage is markedly enhanced in the presence of a catalytic amount of the haem-containing enzyme horseradish peroxidase [89]. Various flavonoids including (−)-epigallocatechin gallate (polyphenol) inhibit peroxynitrite-mediated oxidation and nitration reactions [88, 90, 91].

Vitamin E, the major lipophilic antioxidant, consists of α-, β- and γ-tocopherols. γ-Tocopherol bears an additional methyl group at position 5 of α-tocopherol. Peroxynitrite converts α-tocopherol into tocopherolquinone in nearly quantitative yields [92], whereas γ-tocopherol is nitrated by peroxynitrite [93].

Ebselen and seleno compounds

Ebselen [2-phenyl-1,2-benzisoselenazol-3(2H)-one], an organoselenium compound with glutathione-peroxidase-like activity, reacts with peroxynitrite very efficiently, yielding the corresponding selenoxide as the sole selenium-containing product with a rate constant of 2×10^6 $M^{-1}\,s^{-1}$ [94]. Rate constants of oxidation of D,L-selenomethionine by peroxynitrite are 10- to 1000-fold higher than those for the reactions of methionine with peroxynitrite [95]. Seleno-organic compounds can inhibit oxidation and nitration reactions mediated by peroxynitrite [96].

Glutathione peroxidase protects against peroxynitrite-mediated oxidation and nitration in the presence of glutathione more effectively than ebselen, thus probably acting as a peroxynitrite reductase in vivo to defend against peroxynitrite-induced tissue/DNA damage [97].

Haemoproteins and porphyrin derivatives

The peroxynitrite-mediated oxidation of oxyhaemoglobin is indistinguishable from that obtained with $^{\bullet}NO$. The oxidation rate of ferrocytochrome c by peroxynitrite is estimated to be $1.6 \times 10^5\ M^{-1}\ s^{-1}$, which is comparable to that obtained with myeloperoxidase ($5 \times 10^5\ M^{-1}\ s^{-1}$). Simulation of fluxes of $^{\bullet}NO$ and superoxide in the presence of oxyhaemoglobin and ferricytochrome c, which are able to trap $^{\bullet}NO$ and $O_2^{\bullet -}$, respectively, demonstrates that very little peroxynitrite is formed in the presence of micromolar amounts of haemoproteins [16]. Synthetic iron (III) and manganese (II) porphyrin derivatives react rapidly with peroxynitrite ($\sim 2 \times 10^6\ M^{-1}\ s^{-1}$). 5,10,15,20-Tetrakis(2,4,6-trimethyl-3,5-disulphonatophenyl)-porphyrinato iron (III) is effective against cytotoxicity caused by exogenously added peroxynitrite as well as inflammation induced by carrageenan in rats [98].

Other antioxidants

Reactions of peroxynitrite with ascorbate ($1 \times 10^2\ M^{-1}\ s^{-1}$) and uric acid have been reported [49, 99–101]. Peroxynitrite-mediated haemolysis is effectively inhibited by glutathione, N-acetylcysteine and albumin, while trolox, a water-soluble analogue of tocopherol, and uric acid do not seem to be very effective [102]. CO_2/bicarbonate is an efficient antioxidant against peroxynitrite damage in extracellular fluids. Although glutathione, uric acid and ascorbate do not react efficiently with the peroxynitrite-CO_2 adduct, they are able to scavenge tyrosyl and tryptophanyl radicals which are created by peroxynitrite [49]. $^{\bullet}NO$ itself is a very efficient scavenger of these radicals. Other antioxidants, which have been reported to react with or scavenge peroxynitrite, include bilirubin [103], β-carotene [104] and melatonin [105]. Furthermore, uric acid is converted by peroxynitrite to an unstable vasorelaxant product suggesting a reductive role of urate which is able to produce $^{\bullet}NO$ from peroxynitrite [106].

DNA base modifications

DNA damage induced by peroxynitrite has recently been reviewed [107]. In accordance with other biomolecular targets, DNA is damaged by peroxynitrite through nitration and oxidation. Reactions of isolated DNA with authentic peroxynitrite form 8-nitroguanine dose dependently [108]. Only peroxynitrite, but not nitrous acid, tetranitromethane or $^{\bullet}NO$-releasing compounds, forms 8-nitroguanine. Therefore, 8-nitroguanine in DNA could be measured as a specific marker for peroxynitrite-mediated DNA damage. Bicarbonate (0–10 mM) causes a dose-dependent increase of up to sixfold in the formation of 8-nitroguanine in DNA. The reaction of 2′-deoxyguanosine with peroxynitrite was also shown to yield several compounds, two of which were identified as 4,5-dihydro-5-hydroxy-4-(nitrosooxy)-2′-deoxyguanosine and 8-nitroguanine [109]. The reaction of deoxynucleosides with peroxynitrite has been reported to yield highly cytotoxic base-propenals (base-CH = CH-CHO) [43, 110]. On the other hand, peroxynitrite induces only small increases in some oxidized bases including 8-oxoguanine, FAPy-guanine, 8-oxoadenine and oxazolone [108, 111, 112]. One possible explanation would be that oxidized bases such as 8-oxoguanine may be further oxidized into the ring cleavage product by peroxynitrite [113].

Single-strand breakage can occur in plasmid DNA following treatment with concentrations of peroxynitrite as low as 1 μM [110]. Peroxynitrite induces significantly more single-strand scissions at acidic pH than at neutral or alkaline pH. The presence of CO_2/bicarbonate inhibits the DNA breakage, suggesting that peroxynitrous acid (ONOOH) is responsible for the damage [43]. Concurrent generation of $^{\bullet}NO$ and superoxide can also induce strand breakage under a variety of conditions. 3-Morpholinosydnonimine (SIN-1), that is assumed to generate simultaneously $^{\bullet}NO$ and superoxide, thus possibly forming peroxynitrite, has also been used to study DNA damage. Yermilov et al. [108] have reported that SIN-1 increases dose dependently the level of 8-oxoguanine, but not that of 8-nitroguanine, in DNA, in contrast with the fact that authentic peroxynitrite formed 8-nitroguanine, but not 8-oxoguanine, in DNA. One possible reason for this observation could be that SIN-1 may produce unidentified reactive oxidants, which may be responsible for the formation of 8-oxoguanine [108, 114].

Conclusion

Peroxynitrite is a fascinating molecule and presents multiple facets that account for its complex chemistry which is strictly dependent on the environment. Indeed, the ratio of superoxide to $^{\bullet}NO$ is important in determining the reactivity of peroxynitrite: excess $^{\bullet}NO$ or excess superoxide affects the oxidation and nitration reactions elicited by peroxynitrite [4, 23]. There is now good evidence suggesting that peroxynitrite is formed in vivo and plays an important role in diverse pathophysiological conditions such as inflammation, neurodegenerative diseases and cardiovascular disorders.

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