CHAPTER 3

Mechanisms and Biological Consequences of Peroxynitrite-Dependent Protein Oxidation and Nitration

Madia Trujillo[1,2], Beatriz Alvarez[2,3], José M. Souza[1,2], Natalia Romero[1,2], Laura Castro[1,2], Leonor Thomson[2,3] and Rafael Radi[1,2]

[1]Departamento de Bioquímica

[2]Center for Free Radical and Biomedical Research, Facultad de Medicina, Universidad de la República, Uruguay

[3]Laboratorio de Enzimología, Facultad de Ciencias, Universidad de la República, Uruguay

SUMMARY

Peroxynitrite modifies proteins through different mechanisms. According to kinetic considerations, direct peroxynitrite reactions with proteins *in vivo* are most probably restricted to fast reacting thiols as well as metal cofactors. Other amino acidic or prosthetic group modifications, including tyrosine nitration, require the formation of peroxynitrite-derived secondary oxidants. The interaction between peroxynitrite and proteins can lead to peroxynitrite detoxification, such as peroxiredoxin-catalyzed reduction to nitrite or oxyhemoglobin-mediated isomerization to nitrate. Alternatively, peroxynitrite can promote changes in protein function by oxidation and/or nitration of key protein moieties. Considering the low abundance of modified amino acidic residues, their biological relevance is clearer in the case of the gain of a new function. Peroxynitrite reactions with proteins also promote protein aggregation, turnover, signaling and immunological processes. Herein, we review the biochemical mechanisms of peroxynitrite-dependent protein modifications and analyze the impact on protein function *in vitro* and *in vivo*.

Keywords: peroxynitrite, 3-nitrotyrosine, oxidative stress, sulfenic acid, kinetics, fast reacting thiols, nitric oxide, nitrogen dioxide, superoxide, free radicals

Nitric Oxide: Biology and Pathobiology, second edition

OVERVIEW OF PEROXYNITRITE BIOCHEMISTRY

Since peroxynitrite was first proposed to be implicated in pathophysiology almost two decades ago (Beckman *et al.*, 1990; Ischiropoulos *et al.*, 1992; Radi *et al.*, 1991a,b), scientists from different fields have made important contributions regarding its routes of formation, mechanisms of reaction, detoxification and cellular actions (Ferrer-Sueta and Radi, 2009; Pacher *et al.*, 2007; Szabo *et al.*, 2007). Peroxynitrite is now known to be implicated in an increasing number of diseases, including neurodegenerative disorders (Torreilles *et al.*, 1999), atherogenesis and related cardiovascular diseases (Uppu *et al.*, 2007; Wattanapitayakul *et al.*, 2000), diabetes (Arora *et al.*, 2008; Zou *et al.*, 2004), and immune and inflammatory disorders (Cross *et al.*, 1997; Oates *et al.*, 1999; Sandhu *et al.*, 2003). Understanding the mechanisms by which peroxynitrite exerts its pathogenic actions can assist in the development of rational treatments for such diseases.

The main route of peroxynitrite formation in biological systems is the fast reaction between superoxide anion ($O_2^{\bullet -}$) and nitric oxide ($^{\bullet}NO$) radicals. The rate constant of this reaction is in the 10^9–$10^{10}\,M^{-1}s^{-1}$ range (Goldstein and Czapski, 1995b; Huie and Padmaja, 1993; Kissner *et al.*, 1997). Thus, the reaction between these two radicals is fast enough to outcompete $O_2^{\bullet -}$ dismutation catalyzed by superoxide dismutase (SOD), at least under conditions of increased $^{\bullet}NO$ production, such as inflammation via inducible NO synthase (iNOS) or sustained activation of the constitutive forms of NOS (Nakamura and Lipton, 2008). When considering sites of peroxynitrite formation, the anionic nature of $O_2^{\bullet -}$ at physiological pH ($pK_a\ HO_2 = 4.8$), and hence its limited diffusion capability, should be taken into account. This is not the case for $^{\bullet}NO$, which is a small and neutral lipophilic molecule that can readily diffuse through membranes. Therefore, peroxynitrite formation is favored in those cellular compartments where $O_2^{\bullet -}$ is produced. Other biologically relevant routes for peroxynitrite formation may include the reaction between triplet nitroxyl anion and molecular oxygen, which could occur in selected environments (Shafirovich and Lymar, 2002), as well as metal-bound peroxynitrite formation from the reaction of $^{\bullet}NO$ and ferrous-dioxygen complexes in heme-containing proteins like oxyhemoglobin (Herold, 1998; Romero *et al.*, 2003).

Since peroxynitrous acid has a pK_a of 6.5–6.8 (Goldstein and Czapski, 1995b; Kissner *et al.*, 1997; Pryor and Squadrito, 1995), peroxynitrite predominates as the anion at physiological pH, and hence its diffusion through membranes is limited. In fact, peroxinitrous acid can cross biological membranes by passive diffusion while the anionic species uses anion channels, its permeation being restricted by the number of channels present in the cellular membrane (Denicola *et al.*, 1998; Marla *et al.*, 1997).

Peroxynitrous acid is an unstable species that decays through homolysis of its peroxo bond ($k = 0.9\,s^{-1}$, pH 7.4, 37 °C), resulting in hydroxyl ($^{\bullet}OH$) and nitrogen dioxide ($^{\bullet}NO_2$) radicals in approximately 30% yields (Gerasimov and Lymar, 1999; Goldstein and Czapski, 1995a). Both radicals participate in secondary reactions that may lead to the oxidation/nitration of different targets. Moreover, both peroxynitrite anion and peroxynitrous acid are strong oxidants that *directly* react with different biomolecules. Among these, low molecular weight and protein thiols, metal centers and carbon dioxide (CO_2) constitute the main targets for peroxynitrite *in vivo* (for a recent review see Ferrer-Sueta and Radi, 2009). The mechanisms of oxidation vary depending on the target. The direct reaction with thiols involves peroxynitrous acid as the oxidant species (Trujillo and Radi, 2002) and consists of a two-electron oxidation process (Radi *et al.*, 1991b). On the other hand, peroxynitrite anion reacts with CO_2 ($k = 4.6 \times 10^4\,M^{-1}s^{-1}$ at pH 7.4 and 37 °C) to form a transient intermediate that very rapidly decomposes homolytically to $^{\bullet}NO_2$ and carbonate radical ($CO_3^{\bullet -}$) in ~ 35% yields (Bonini *et al.*, 1999; Denicola *et al.*, 1996; Lymar and Hurst, 1995). These two radicals can in turn lead to secondary or *indirect* reactions. In the case of metal centers, such as heme proteins or manganese porphyrins, reported mechanisms include one-electron and two-electron oxidations as well as metal-catalyzed isomerization to nitrate (Ferrer-Sueta *et al.*, 1999; Floris *et al.*, 1993; Romero *et al.*, 2003; Thomson *et al.*, 1995; Zou *et al.*, 1999a).

To rationalize the biological fate of peroxynitrite and its derived radicals, it is necessary to understand the kinetics of the reactions. In this regard, many direct reactions of peroxynitrite have been addressed kinetically, with reported rate constants (k) ranging from $10^2\,M^{-1}s^{-1}$ to $10^8\,M^{-1}s^{-1}$, and reactions of $^{\bullet}OH$, $^{\bullet}NO_2$ and $CO_3^{\bullet-}$ have been studied as well. It should be taken into account, importantly, that the main targets for peroxynitrite will be dictated not by rate constants but by overall reaction rates, which are determined by the product of rate constant and biological target concentration (k′). Care should be taken in determination of the rate constants of peroxynitrite reactions, particularly when stopped-flow methods are not available and competition approaches are used (Trujillo *et al.*, 2008a). Most common misinterpretations arise from the fact that peroxynitrite-derived radicals can also lead to target oxidation, and therefore the use of radical scavengers that react with peroxynitrite-derived radicals but not directly with peroxynitrite itself is highly recommended. Moreover, buffers such as HEPES, MOPS, and Tris, which have been reported to react with peroxynitrite-derived radicals and form products with their own reactivity, should be avoided (Gadelha *et al.*, 1997; Schmidt *et al.*, 1998). Since peroxynitrite anion diffusion through membranes is limited (Denicola *et al.*, 1998; Marla *et al.*, 1997), sites of peroxynitrite formation, diffusion capability and target compartmentalization should also be considered to understand its biological fate. Moreover, the radical nature of peroxynitrite precursors as well as products formed from its decomposition or one-electron oxidations, and the multiple possible interactions among them, add an additional level of complexity.

Peroxynitrite-mediated protein oxidation and nitration have been shown to be biologically relevant processes, leading in many cases to altered protein function, which not only includes protein inactivation but also activation or, most importantly, gain of a new function (for recent reviews see Peluffo and Radi, 2007; Souza *et al.*, 2008b). In some cases, the modification caused by peroxynitrite leads to protein aggregation (Souza *et al.*, 2000b; Zhang *et al.*, 2005b) or degradation (Grune *et al.*, 2001; Souza *et al.*, 2000a). Peroxynitrite has also been reported to be involved in cell signaling processes (Huang *et al.*, 2008). More recently, peroxynitrite-modified proteins have been demonstrated to be immunogenic and have been implicated in the development of inflammatory diseases (Thomson *et al.*, 2007). Finally, the interaction of peroxynitrite with proteins specialized towards its catalytic decomposition results in peroxynitrite detoxification without a net protein modification, either because these proteins catalyze peroxynitrite isomerization (Romero *et al.*, 2003), or as a result of the rapid re-reduction of oxidized protein intermediates by other cellular compounds (Trujillo *et al.*, 2008b).

In this chapter we will present the mechanisms of reaction between peroxynitrite and amino acid residues as well as prosthetic groups in proteins. Selected examples of such protein modifications and their biological consequences will be analyzed.

PEROXYNITRITE-MEDIATED AMINO ACID AND PROTEIN MODIFICATIONS

Although virtually all amino acids would be modified if exposed to high enough concentrations of peroxynitrite, its direct reactivity is restricted to cysteine, selenocysteine, methionine, and tryptophan residues, as well as metal prosthetic groups in proteins.

Cysteine oxidation

Direct peroxynitrite-mediated thiol oxidation is a two-electron process in which peroxynitrous acid oxidizes thiolates to the corresponding sulfenic acids (RSOH) (Radi *et al.*, 1991b) (Fig. 1). The latter are unstable species that in the presence of accessible thiols form disulfides (RSSR′), within the same or other proteins, or with low molecular weight thiols, leading to mixed disulfide formation. The stoichiometry of this reaction is therefore two thiols oxidized for each peroxynitrite. In selected cases sulfenic acids are stable, and then

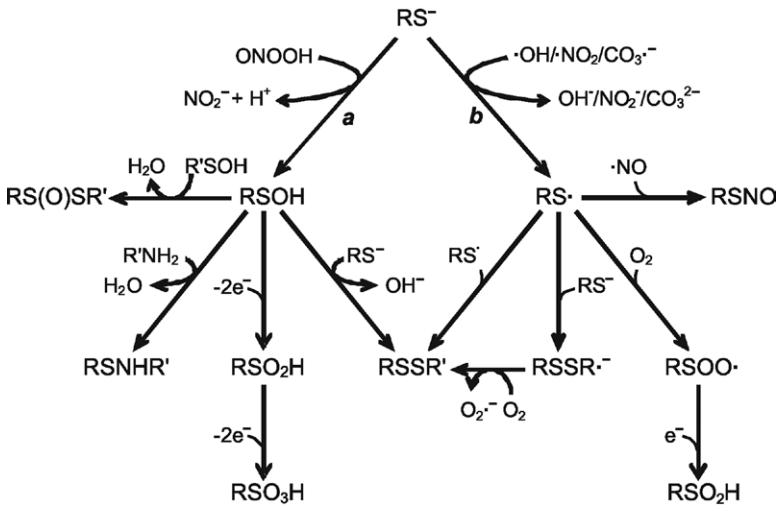

FIGURE 1

Possible reactions of thiols exposed to peroxynitrite. To the left (***a***) are depicted two-electron mechanisms. Thiolates (RS^-) react with peroxynitrous acid forming sulfenic acid (RSOH). The latter can react with another thiol forming a disulfide (RSSR'). Alternatively, sulfenic acid can react with itself forming a thiosulfinate (RS(O)R'), or with an amine forming a sulfenamide (RSNHR'). Sulfenic acid can be further oxidized by two electrons forming sulfinic (RSO_2H) and sulfonic (RSO_3H) acid. To the right (***b***) are depicted one-electron mechanisms. Thiolates (RS^-) react with the radicals derived from peroxynitrite forming the thiyl radical ($RS^\bullet$), which can react with itself forming a disulfide or with $^\bullet NO$ forming a S-nitrosothiol (RSNO). The thiyl radical can also react with dioxygen, eventually leading to sulfinic acid formation, or with another thiolate. The latter reaction gives rise to the disulfide anion radical ($RSSR^{\bullet-}$), a reductant that can react with dioxygen forming superoxide and a disulfide (RSSR).

yields of thiol oxidation are lower (1:1) (Allison *et al.*, 1973; Carballal *et al.*, 2003; Claiborne *et al.*, 1993). Alternatively, sulfenic acid can dismutate to thiosulfinate (RS(O)SR') or react with an amine or amide forming a sulfenamide (RSNHR'). Disulfides, sulfenic acid and sulfenamides are modifications that can be reverted by the addition of further thiol. In the presence of excess peroxynitrite or other two-electron oxidants, sulfenic acid can be further oxidized to sulfinic and sulfonic acids (RSO_2H and RSO_3H; Radi *et al.*, 1991b). Indeed, irreversible oxidation of protein thiols by peroxynitrite has been described (Konorev *et al.*, 1998; Takakura *et al.*, 1999). The formation of sulfinic acid has usually been considered an irreversible modification. Nevertheless, enzymatic mechanisms for sulfinic acid reduction for specific proteins have been described (Biteau *et al.*, 2003; Georgiou and Masip, 2003; Rabilloud *et al.*, 2002; Rhee *et al.*, 2005).

In addition to the direct reaction, peroxynitrite-derived radicals, $^\bullet OH$, $^\bullet NO_2$ and $CO_3^{\bullet-}$ can lead to indirect thiol oxidation, which consists of a one-electron oxidation process to the corresponding thiyl radicals (Bonini and Augusto, 2001; Quijano *et al.*, 1997). These radicals may recombine to form disulfide bridges. More frequently, thiyl radicals react with oxygen to form thiylperoxyl radicals ($RSOO^\bullet$) that can rearrange to sulfonyl radicals ($RSO_2^\bullet$) and subsequently sulfinic/sulfonic acid, or further react with another thiol eventually leading to sulfenic acid (Wardman, 1998). Thiyl radicals can also react with thiols to form disulfide anion radicals, which in turn can reduce oxygen to form superoxide and disulfide anion radicals ($RSSR^{\bullet-}$) (Wardman and von Sonntag, 1995). Moreover, thiyl radicals react at diffusion-controlled rates with nitric oxide to form S-nitrosothiols (RSNO) (Madej *et al.*, 2008). The latter can also be formed from the reaction of thiols with dinitrogen trioxide (N_2O_3) derived from the fast reaction between peroxynitrite-derived $^\bullet NO_2$ and $^\bullet NO$ (Kharitonov *et al.*, 1995). Therefore, products formed from peroxynitrite-mediated thiol oxidation may differ according to the mechanism of reaction, depending on the concentration of thiol, oxygen and carbon dioxide, and on whether proteins are exposed to peroxynitrite as a bolus (no $^\bullet NO$ is present) or generated by fluxes of precursor radicals.

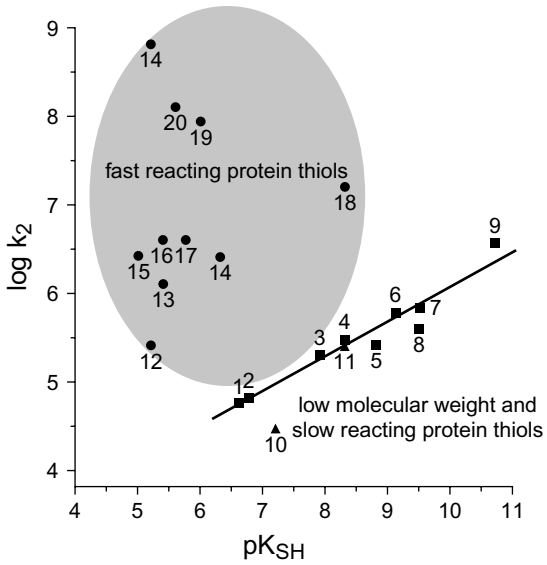

FIGURE 2

Kinetics of the peroxynitrite reduction by thiols. Intrinsic thiolate reactivities (k_2, pH independent), calculated from pH-dependent rate constants (Table 1) using Eq. 1, were plotted against thiol pK_a (pK_{SH}). Low molecular weight thiols (squares, 1–9) show a positive Brønsted correlation, as indicated by the line, consistent with the thiols with higher pK_a being better nucleophiles. Some protein thiols (triangles, 10 and 11) react with peroxynitrite as expected according to their thiol pK_a. Other protein thiols (circles, 12–21), react much faster than expected, indicating that protein factors others than thiol pK_a are determining this reactivity. 1. Cysteine ethyl ester, 2. cysteine methyl ester, 3. penicillamine, 4. cysteine, 5. glutathione, 6. mercapto ethyl guanidine, 7. homocysteine, 8. N-acetyl cysteine, 9. dihydrolipoic acid, 10. *Trypanosoma brucei* tryparedoxin, 11. human serum albumin, 12. human arylamine N-acetyltransferase 1, 13. DJ1, 14. TSA2, 15. *Mycobacterium tuberculosis* AhpC, 16. creatine kinase, 17. TSA1, 18. GAPDH, 19. red blood cell Prx2, 20. PTP1B, 21. human Prx5. Modified from Trujillo *et al.* (2007) with permission from Elsevier.

The kinetics of direct peroxynitrite-mediated thiol oxidation have been determined for several protein and low molecular weight thiols (Fig. 2). The pH dependencies of the rate constants are bell-shaped, according to:

$$k_{2app} = k_{2pHind}\left(\frac{[H^+]}{K_a^{ONOOH} + [H^+]}\right)\left(\frac{K_a^{RSH}}{K_a^{RSH} + [H^+]}\right) \quad (1)$$

where k_{2app} is the apparent rate constant at a given pH, k_{2pHind} is the pH independent rate constant, i.e. the intrinsic thiolate reactivity, and K_a^{ONOOH} and K_a^{RSH} are the dissociation constants of ONOOH and RSH, respectively (Radi *et al.*, 1991b; Trujillo and Radi, 2002).

For many thiols, including low molecular weight thiols and some protein thiols such as the single thiol group of human serum albumin (HSA), reactivities are related to thiol pK_a, those thiols with the lower pK_a reacting faster at pH 7.4 due to increased thiolate fraction (Trujillo and Radi, 2002). On the contrary, intrinsic thiolate reactivity increases with lower thiol pK_a reflecting changes in thiolate nucleophilicity, on agreement with a reaction mechanism involving the nucleophilic attack of the thiolate on the peroxidic oxygen of peroxynitrite with nitrite as leaving group (Trujillo *et al.*, 2007). However, there are some protein thiols whose reactivities with peroxynitrite are much higher than expected from their pK_a. These fast reacting thiols include the peroxidatic thiol in peroxiredoxins, and those of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (Souza and Radi, 1998) and protein tyrosine phosphatases PTP1B (Takakura *et al.*, 1999) (Fig. 2). It is obvious that in these cases, protein factors other than the thiol pK_a are responsible for this fast reactivity (Trujillo *et al.*, 2007). Further work is required to unravel these factors.

HUMAN SERUM ALBUMIN

HSA is the most abundant protein in the intravascular space (~0.6 mM), constituting 60% of total plasma protein, and is also present extravascularly in extracellular tissue compartments. It contains only one free cysteine, Cys34 (HSA-SH), which is the main plasma thiol, representing > 80% of free thiols in the circulation (Peters, 1996). It has been proposed to participate in the scavenging of oxidant species, mostly due to the presence of the Cys34 thiol. Indeed, HSA-SH is able to react with different oxidants, including peroxynitrite and its derived radicals. The reaction with peroxynitrite occurs with a second order rate constant of $3.8 \times 10^3\,M^{-1}s^{-1}$ (Alvarez *et al.*, 1999). This reactivity is comparable to that of low molecular weight thiols such as free cysteine ($k = 5.9 \times 10^3\,M^{-1}s^{-1}$; Radi *et al.*, 1991b) and glutathione ($k = 1.35 \times 10^3\,M^{-1}s^{-1}$; Koppenol *et al.*, 1992), and is consistent with the apparent pK_a value of the albumin thiol of 8.3–8.6 (Alvarez *et al.*, 1999), as illustrated in Fig. 2. The reaction with two-electron oxidants leads to a relatively stable sulfenic acid (HSA-SOH) (Carballal *et al.*, 2003), which can react with a thiol such as cysteine leading to a mixed disulfide (HSA-SSR) or be further oxidized to sulfinic ($HSA\text{-}SO_2H$) and sulfonic ($HSA\text{-}SO_3H$) acid. No evidence has been obtained for the formation of sulfenamides or intermolecular disulfide dimers. A recent analysis of the reactivity of albumin sulfenic acid (Turell *et al.*, 2008) supports quantitatively a role for this intermediate in the formation of mixed disulfides and higher oxidation states, which can be observed in ~25% of circulating albumin.

In addition to the direct reaction with peroxynitrite, HSA-SH also reacts with the radicals derived from peroxynitrite homolysis, $^{\bullet}OH$ ($k = 3.8 \times 10^{10}\,M^{-1}s^{-1}$; Goldstein and Samuni, 2005), $^{\bullet}NO_2$ and $CO_3^{\bullet-}$ ($k = 5.8 \times 10^8\,M^{-1}s^{-1}$; Goldstein and Samuni, 2005), leading to thiyl radical and other secondary radicals (Gatti *et al.*, 1994). Although the albumin thiol does not react particularly fast with oxidants, it can still be considered an important intravascular scavenger due to its very high concentration, particularly considering that plasma is scarce in antioxidant defenses and low molecular weight thiols. In this regard, oxidized isoforms of albumin have been found to be increased in several pathological conditions (for a review see Turell *et al.*, 2009). These oxidized species are not present when albumin is secreted from liver cells. Thus, they can be considered potential biomarkers of the involvement of oxidative stress processes and constitute a proof of the concept of the scavenger activity of albumin.

PEROXIREDOXINS

Peroxiredoxins (Prxs) are ubiquitous peroxidases that rely on active site Cys residue(s) to reduce peroxides (Hofmann *et al.*, 2002; Wood *et al.*, 2003). The first step in their catalytic cycle, common for all kinds of Prxs, consists of the oxidation of the peroxidatic cysteine to a sulfenic acid derivative. In one-Cys Prxs, reduction back to thiol is achieved directly by the reducing substrate, whose nature is still controversial (Monteiro *et al.*, 2007; Ralat *et al.*, 2006). In two-Cys Prxs, a second (resolving) cysteine residue forms a disulfide bridge with the sulfenic acid in the peroxidatic residue, which in turn is reduced by thioredoxin/thioredoxin reductase at the expense of NADPH (Wood *et al.*, 2003). In 2000, Bryk *et al.* reported the peroxynitrite-reductase activity of bacterial alkyl hydroperoxide reductase C (AhpC), a typical two-Cys Prx. Afterwards, peroxynitrite reduction to nitrite was demonstrated to be a general property of all kinds of Prxs studied so far (Table 1), which include examples of the different classes of Prx present in microorganisms, plants and animals (Dubuisson *et al.*, 2004; Jaeger *et al.*, 2004; Manta *et al.*, 2008; Nickel *et al.*, 2005; Ogusucu *et al.*, 2007; Peshenko *et al.*, 2001; Sakamoto *et al.*, 2003; Trujillo *et al.*, 2004). The peroxidatic thiol pK_a in Prxs have been reported to be in the 5–6.3 range for the different Prxs already studied, indicating that it would be mostly (>90%) deprotonated at physiological pH (Bryk *et al.*, 2000; Nelson *et al.*, 2008; Ogusucu *et al.*, 2007; Trujillo *et al.*, 2007). However, the kinetics of peroxynitrite reduction are much faster than expected according to peroxidatic thiol pK_a (Fig. 2) (Trujillo *et al.*, 2007). The protein factors generating such a fast reactivity are intriguing. As with other peroxide substrates, peroxynitrite-mediated peroxidatic thiol oxidation leads to sulfenic acid

Table 1 Selected examples of peroxynitrite-mediated amino acid modifications in proteins and their biological consequences

Residue	Protein	Rate constant ($M^{-1}s^{-1}$)[a]	Modification	Position	Biological consequence	Reference
Cysteine	Bovine peroxiredoxin 6 (1-Cys Prx)	NR	Sulfenic acid	Peroxidatic Cys (Cys47)	Peroxynitrite catalytic reduction[b]	(Peshenko *et al*., 2001)
	2-Cys peroxiredoxins (typical)	10^6–10^7	Intermolecular disulfide	Peroxidatic Cys	Peroxynitrite catalytic reduction[c]	(Bryk *et al*., 2000; Manta *et al*., 2008[d]; Nickel *et al*., 2005; Trujillo *et al*., 2004)
	2-Cys peroxiredoxins (atypical)	10^7–$10^{8,d}$	Intramolecular disulfide	Peroxidatic Cys	Peroxynitrite catalytic reduction[c]	(Dubuisson *et al*., 2004; Jaeger *et al*., 2004; Trujillo *et al*., 2007)
	Protein tyrosine phosphatases	$2–22 \times 10^7$	Sulfinic or sulfonic acid	Active site Cys	Protein inactivation	(Takakura *et al*., 1999)
	Human arylamine N-acetyltransferase 1	5×10^4	Mostly sulfinic or sulfonic acid	Cys68	Protein inactivation	(Dupret *et al*., 2005)
	Rabbit glyceraldehyde-3-phosphate dehydrogenase	$2.5 \times 10^{5,d}$	85% sulfinic or sulfonic acid	Cys149	Protein inactivation	(Souza and Radi, 1998)
	DJ-1	$2.7 \times 10^{5,d}$	Sulfinic acid	Cys106	Protein inactivation Possible gain of chaperone activity	(Andres-Mateos *et al*., 2007)
	Rabbit creatine kinase	$8.8 \times 10^{5,e}$	Sulfinic or sulfonic acid	Cys283	Protein inactivation	(Konorev *et al*., 1998)
	Human albumin thiol	3.8×10^3	Sulfenic acid	Cys34	Not clear change in protein function	(Alvarez *et al*., 1999)
	Sarco/endoplasmic reticulum calcium ATPase	NR	S-glutathiolation	Cys674	Increased activity	(Adachi *et al*., 2004)

(*Continued*)

Table 1 Continued

Residue	Protein	Rate constant ($M^{-1}s^{-1}$)[a]	Modification	Position	Biological consequence	Reference
	Aldose reductase	NR	Sulfenic acid	Cys298	Increased activity	(Kaiserova *et al*., 2008)
	Tau and microtubule-associated protein-2	NR	Disulfide formation	NR	Inhibited microtubule polymerization	(Landino *et al*., 2004)
Selenocysteine	Glutathione peroxidase	8×10^6	Seleninic acid	Sec47	Peroxynitrite catalytic reduction	(Briviba *et al*., 1998)
Methionine	α1-Proteinase inhibitor	NR	MetSO	Met358	Protein inactivation	(Moreno and Pryor, 1992)
	Calmodulin	NR	MetSO	Met144–Met145	Protein inactivation	(Galeva *et al*., 2005)
Tryptophan and histidine	Succinyl CoA : 3 ketoacid transferase	NR	5-hydroxy 6-nitroTrp	Trp342	Increased activity	(Rebrin *et al*., 2007)
	Human CuZn superoxide dismutase	$1 \times 10^{4,f}$	His oxidation ($-CO_2$) 6-nitroTrp ($+CO_2$)	His118 Trp32	Inactivation	(Alvarez *et al*., 2004; Yamakura *et al*., 2005)
Tyrosine	Human Mn superoxide dismutase	$1 \times 10^{5,g}$	3-nitroTyr	Tyr34	Protein inactivation	(MacMillan-Crow *et al*., 1998)
	Prostacyclin synthase	10^6–$10^{7,h}$	3-nitroTyr	Tyr430	Protein inactivation	(Schmidt *et al*., 2003; Zou, 2007)
	Microsomal glutathione S-transferase	NR	3-nitroTyr	Tyr92	Increased activity[i]	(Ji *et al*., 2006)
	Cytochrome c^{3+}	ND	3-nitroTyr	Tyr97 or Tyr74[j]	Increased peroxidase activity. Inhibition of electron transport and apoptosome assembly. Translocation to cytosol.	(Batthyany *et al*., 2005; Cassina *et al*., 2000; Gebicka and Didik, 2003; Souza *et al*., 2008a, and Godoy *et al*., 2008)

	Fibrinogen	NR	3-nitroTyr	Tyr292, Tyr422 of beta-chain[k]	Acceleration in fibrin clot formation	(Vadseth *et al*., 2004)
	Human glutathione reductase	NR	3-nitroTyr	Tyr106, Tyr114	Protein inactivation	(Savvides *et al*., 2002)
	Nerve growth factor	NR	3-nitroTyr	Tyr 52[l]	Increased apoptotic activity	(Pehar *et al*., 2006)
	Cytochrome P450 2B1	NR	3-nitroTyr	Tyr190	Protein inactivation	(Lin *et al*., 2003)

[a]At pH 7.4 and 37°C unless otherwise indicated.

[b]Two physiologically relevant routes for reduction of oxidized enzyme have been described: heterodimerization with glutathione transferase that allows glutathione-mediated reduction (Ralat *et al*., 2006) and ascorbate-dependent reduction (Monteiro *et al*., 2007).

[c]Catalytic cycle is complete at thioredoxin (or thioredoxin-related protein)/thioredoxin reductase/NADPH expense.

[d]At 25°C;

[e]at pH 6.9.

Kinetic data refers to the global reaction between peroxynitrite and the proteins, including the metal cofactors:

[f]reaction with copper cofactor, indirectly leads to histidinyl radical formation;

[g]with the manganese cofactor, that indirectly leads to tyrosine nitration. Dityrosine formation is also observed;

[h]reaction with heme thiolate cofactor, indirectly leading to tyrosine nitration.

[i]Nitration at Tyr92 is responsible for the gain of function. Tyr153 nitration and thiol oxidation are also observed.

[j]At higher concentrations of peroxynitrite, dinitrated species (at Tyr97 and Tyr67 or at Tyr74 and Tyr67) and trinitrated species (at Tyr97, 67 and 74) are observed.

[k]Identified as site of fibrinogen nitration *in vivo* (Parastatidis *et al*., 2008).

[l]Trp99 is also nitrated by peroxynitrite, but protein inactivation has been ascribed to tyrosine nitration.

NR = not reported, ND = not detected.

formation, which is in turn reduced by thioredoxin or other reducing agents. However, when peroxynitrite is in excess, over-oxidation of the peroxidatic thiol to sulfinic acid (Peshenko *et al.*, 2001), as well as tyrosine nitration and dimerization, have also been reported (Manta *et al.*, 2008). The functional significance of the latter processes is still to be demonstrated.

The importance of Prxs for peroxynitrite detoxification at the cellular level has been confirmed in several systems. In *Saccharomyces cerevisiae* there exist five Prxs, including the thiol-specific antioxidant enzymes TSA1 and TSA2. Yeasts deficient in these two proteins were hypersensitive to peroxynitrite, and the phenotype was rescued by the expression of either the *tsa1* or *tsa2* gene (Wong *et al.*, 2002). In *Trypanosoma cruzi*, both cytosolic and mitochondrial Prxs efficiently decomposed exogenous and endogenously generated peroxynitrite in intact cells, and protective effects were lost by mutation of the peroxidatic cysteine residues in both enzymes (Piacenza *et al.*, 2008). Peroxiredoxins present in *Leishmania chagasi* also increased survival during the oxidative stress associated with infection (Barr and Gedamu, 2003). Moreover, mitochondrial Prx 3 protected hippocampal neurons from excitotoxic injury *in vivo* and decreased associated protein tyrosine-nitration (Hattori *et al.*, 2003). An interplay between $^{\bullet}NO$ and Prxs, by which $^{\bullet}NO$ modulates both redox state and expression of these enzymes, has been proposed (Abbas *et al.*, 2008; Diet *et al.*, 2007).

Selenocysteine oxidation

Selenium-containing compounds have also been reported to react directly with peroxynitrite (Masumoto and Sies, 1996). Most probably, the reacting species include selenolate (RSe^-) and peroxynitrous acid. As for thiols, peroxynitrite-mediated selenol oxidation is a two-electron oxidation process yielding nitrite and seleninic acid (RSeOH) which in turn reacts with thiols completing the catalytic cycle.

GLUTATHIONE PEROXIDASE I

Reduced glutathione peroxidase type I (GPx I) from bovine erythrocytes, which is a tetrameric enzyme, reacts with peroxynitrite with a second order rate constant of $8 \times 10^6 M^{-1}s^{-1}$ at pH 7.4 and 25°C, and the resulting oxidized form of the enzyme is reduced by the glutathione–glutathione reductase system at the expense of NADPH (Briviba *et al.*, 1998). The concentration of GPx can reach micromolar values in cellular systems (Arteel *et al.*, 1999); therefore, the enzyme should be regarded as a possible target for peroxynitrite, at least in those tissues with higher GPx expression (i.e. 2 µM), since the product of rate constant times concentration ($k' = 16\,s^{-1}$, 25°C) is considerable and would be twice as high at 37°C (Trujillo *et al.*, 2008b). Since k′ for the reaction between peroxynitrite and CO_2 is even higher in most cellular compartments ($\sim 60\,s^{-1}$), research concerning potential reactions between $^{\bullet}NO_2/CO_3^{\bullet -}$ and selenol-containing compounds would be of interest.

The *in vivo* role of GPx in protection against peroxynitrite-mediated cytotoxicity is controversial: although experimental evidence towards a protective role exists, it is scarce (Jozsef and Filep, 2003), and animal models have failed to demonstrate such a role. On the contrary, GPx increased apoptosis induced by peroxynitrite in murine hepatocytes (Fu *et al.*, 2001a,b).

Further studies are required to determine whether other GPx types, either selenium or thiol dependent, can directly react with peroxynitrite. Moreover, other selenium-containing proteins, such as thioredoxin reductase and selenoprotein P have also been reported to react with peroxynitrite (Sies and Arteel, 2000), but mechanistic and kinetic studies for those reactions are still to be addressed.

Methionine oxidation

Peroxynitrite oxidizes methionine residues in proteins either by direct or indirect mechanisms. Direct peroxynitrite-mediated methionine oxidation is a two-electron process leading to

methionine sulfoxide formation, for which enzymatic routes of reduction involving methionine sulfoxide reductases have been described (Fomenko *et al.*, 2008; Kim and Gladyshev, 2005; St John *et al.*, 2001). The reaction between peroxynitrite and free methionine is quite slow ($3.6 \times 10^2\,M^{-1}s^{-1}$ at 37 °C and pH 7.4; Alvarez *et al.*, 1999; Pryor *et al.*, 1994), and the existence of protein factors that could result in increased peroxynitrite direct reactivity towards methionine residues in proteins has not been addressed. According to Perrin and Koppenol (2000), the reaction is much faster with peroxynitrous acid than with peroxynitrite anion (1700 vs. 8.6 $M^{-1}s^{-1}$), and peroxynitrite is mainly reduced to nitrite, although isomerization to nitrate also occurs. Peroxynitrite-derived radicals can oxidize methionine by a one-electron mechanism finally leading to ethylene (Pryor *et al.*, 1994). In the case of methionine residues in peptides or proteins, intramolecular electron transfer reactions from the initial methionine radical cation to other amino acid, namely cysteine, have been proposed (Zhang *et al.*, 2008).

ALPHA 1-ANTITRYPSIN

Peroxynitrite inactivates alpha 1-antitrypsin, the most abundant extracellular anti-protease in the lung, by oxidizing the methionine residue in the inhibitory active site to methionine sulfoxide (Moreno and Pryor, 1992). Kinetic data for this reaction, that could help to rationalize the importance of this enzyme as a target for peroxynitrite, are lacking. Other enzymes that have been reported to form methionine sulfoxide upon addition of peroxynitrite include bacterial glutamine synthetase in the absence of CO_2, whereas in its presence tyrosine nitration is instead the main protein modification observed (Tien *et al.*, 1999). Peroxynitrite-mediated calmodulin oxidation led to methionine sulfoxide formation at different positions (Galeva *et al.*, 2005; Smallwood *et al.*, 2003), although tyrosine nitration also occurred (Table 1).

Tryptophan oxidation

Peroxynitrous acid reacts with tryptophan directly ($k = 37\,M^{-1}s^{-1}$, pH 7.4, 37 °C; Alvarez *et al.*, 1996). This direct reaction is not very fast. Therefore, unless accelerated by protein environment, oxidation of tryptophan in most biological compartments would be an indirect process, produced by peroxynitrite-derived radicals. Carbonate radicals react with tryptophan residues faster than with other amino acid residues in proteins ($k = 10^8\,M^{-1}s^{-1}$ vs $10^7\,M^{-1}s^{-1}$ with cysteine or tyrosine residues; (Chen and Hoffman, 1973). Hydroxyl radicals and $^{\bullet}NO_2$ are also able to oxidize tryptophan to its radical. In fact, the formation of this radical has been detected by EPR (Pietraforte and Minetti, 1997). The recombination with $^{\bullet}NO_2$ leads to tryptophan nitration, mainly 6-nitrotryptophan, although other nitrated isomers and oxidation products are also formed (Alvarez *et al.*, 1996; Yamakura and Ikeda, 2006). It has been postulated that introduction of a nitro group ($-NO_2$) to the 6- or other positions of Trp residues in proteins could affect the structural role of that Trp in a protein by increasing the bulk at this position and/or affect the electron state of the indole ring through the attractive effect of the nitro group (Yamakura *et al.*, 2005). An antibody against 6-nitrotryptophan allowing its detection in selected proteins, as well as in cellular systems, has been developed (Ikeda *et al.*, 2007).

The formation of a hydroxyl-nitro derivative of tryptophan (5-hydroxy-6-nitrotryptophan) has been shown to occur *in vivo* in succinyl-CoA:3-oxoacid CoA transferase (SCOT), in rat heart mitochondria. Modified SCOT has been shown to associate with an elevation of its activity and to accumulate progressively with age (Rebrin *et al.*, 2007).

HUMAN CuZnSOD

CuZnSOD directly reacts with peroxynitrite with a k of $1 \times 10^4\,M^{-1}s^{-1}$ at pH 7.5 and 37 °C, leading to complete protein inactivation (Alvarez *et al.*, 2004). From the pH profile of enzymatic inactivation, peroxynitrite anion was suggested as the oxidizing species. The mechanism of the reaction involves reaction at the copper center of the enzyme active site, forming

$^{\bullet}NO_2$ and a strong oxidant, possibly a highly oxidizing hydroxyl radical (free or bound to the active site cupric ion), which in turn oxidizes a neighboring histidine residue, as was detected by EPR (Alvarez *et al.*, 2004). The enzyme was also inactivated when exposed to simultaneous fluxes of $O_2^{\bullet-}$ and $^{\bullet}NO$, even when flux ratios were varied (Demicheli *et al.*, 2007). In the presence of physiological concentrations of CO_2, however, tryptophan nitration is the main protein modification observed. This is in agreement with CO_2 being a preferential target for peroxynitrite at physiologically relevant concentrations of CO_2 and CuZnSOD, leading to $CO_3^{\bullet-}$ and $^{\bullet}NO_2$ formation, and thereby re-directing the oxidation towards the single tryptophan residue in the enzyme, which results in only a slight decrease in enzymatic activity (Yamakura *et al.*, 2005). In addition, CuZnSOD has been shown to increase yields of peroxynitrite-mediated tyrosine nitration *in vitro*, most probably by reaction of the oxidizing species formed at the level of cupric ion with tyrosine residues followed by recombination with $^{\bullet}NO_2$ (Crow *et al.*, 1997; Ischiropoulos *et al.*, 1992). The role of the enzyme in promoting protein nitration *in vivo* was suggested by the diminished hepatic protein nitration mediated by acetaminophen or lipopolysaccharide in CuZnSOD−/− mice (Jian-Hong *et al.*, 2008). Mutations in CuZnSOD have been implicated in familial amyotrophic lateral sclerosis through the gain of a new and toxic function, either the promotion of oxidative processes or the induction of protein aggregation. It has been proposed that mutated forms of CuZnSOD have a decreased affinity for zinc ion, leading to increased superoxide and hence peroxynitrite formation (Beckman *et al.*, 2001; Estevez *et al.*, 1999).

Reactions with metal-containing protein cofactors

Peroxynitrite can directly oxidize transition metals contained in proteins and low molecular weight compounds, particularly those containing heme and non-heme iron, copper and manganese ions, with rates ranging from 10^4 to $10^7\,M^{-1}s^{-1}$ (Table 2).

The oxidation of the metal center can occur through a one-electron process that results in peroxynitrite reduction to $^{\bullet}NO_2$, or through a two-electron process leading to nitrite (NO_2^-). Moreover, some metal-containing proteins catalyze peroxynitrite isomerization to nitrate (NO_3^-). A picture of these different reactions is shown in Fig. 3, which illustrates heme protein oxidations by peroxynitrite.

In the case of the hemeproteins myeloperoxidase and cytochrome P450, reaction with peroxynitrite leads to ferryl-oxo compounds as intermediates plus $^{\bullet}NO_2$ (Daiber *et al.*, 2000; Floris *et al.*, 1993; Furtmuller *et al.*, 2005). These constitute secondary oxidizing species that can react with exogenous or endogenous targets. Reaction with sacrificial reductants such as ascorbic acid can regenerate the metal center and nitrite, and in such cases the metalloprotein can be considered to catalytically reduce peroxynitrite. In contrast, reaction of the secondary oxidants with critical amino acids nearby may lead to loss of function of the protein. Accordingly, reaction with tyrosine residues, together with the formation of $^{\bullet}NO_2$, is the basis of metal-catalyzed tyrosine nitration, as will be illustrated by the example of MnSOD, where the initial reaction of peroxynitrite with the metal center leads to the site-specific nitration of nearby tyrosine residues. A similar mechanism is responsible for peroxynitrite-mediated prostacyclin synthase inhibition, involving specific nitration of Tyr430, which is close to the iron-thiolate center of the enzyme (Schmidt *et al.*, 2003; Zou, 2007). Metal-oxo species of transition metals in proteins may alter other amino acids, as indicated above for peroxynitrite-mediated histidine oxidation in CuZnSOD. Similarly, peroxynitrite has been postulated as an alternative oxygen donor for the hydroxylation of proline in hypoxia inducible factor 1α under low oxygen availability, through a series of reactions involving the oxidation of the ferric iron of prolyl hydroxylases (Sumbayev and Yasinska, 2006).

Coordination is critical in modulating heme reactivity towards peroxynitrite. In contrast to penta-coordinated globins, peroxidases and Cyt P450, hexa-coordinated heme proteins such as cytochrome c (Gebicka and Didik, 2003; Thomson *et al.*, 1995) and neuroglobin (Herold *et al.*,

Table 2 Mechanisms and kinetics of the reactions of metal-containing proteins with peroxynitrite

Protein	k ($M^{-1}s^{-1}$)	Conditions	Products	Reference
Heme proteins				
Oxyhemoglobin	1.7×10^4	37°C, pH 7.4	Methemoglobin, $O_2{\cdot}^-$ and NO_3^-; low yields of $O = Fe^{IV}$ Hb	(Romero and Radi, 2005)
Cytochrome c^{2+}	2.3×10^5	25°C, pH independent	Fe^{III} cytochrome c and $\cdot NO_2$	(Thomson *et al*., 1995)
Cytochrome c^{3+}	ND[c]			(Gebicka and Didik, 2003)
Myeloperoxidase (Fe^{II})	1.3×10^6	25°C, pH 7	Compound II and NO_2^-	(Furtmuller *et al*., 2005)
Myeloperoxidase (Fe^{III})	2×10^7	12°C, pH independent	Compound II and $\cdot NO_2$	(Floris *et al*., 1993)
	6.8×10^6	25°C, pH 7	Compound II and $\cdot NO_2$	(Furtmuller *et al*., 2005)
Lactoperoxidase	3.3×10^5	25°C, pH 7.4	Compound II and $\cdot NO_2$	(Floris *et al*., 1993)
Horseradish peroxidase	3.2×10^6	25°C, pH independent	Compound I and NO_2^-	(Floris *et al*., 1993)
Catalase	ND[c]			(Floris *et al*., 1993)
Cytochrome c oxidase	$> \sim 10^6$	RT[c], pH 7.4	Two-electron oxidation and NO_2^-	(Pearce *et al*., 1999)
Metmyoglobin	7.7×10^4	20°C, pH 7.0	Metmyoglobin and NO_3^-	(Herold and Shivashankar, 2003)
Methemoglobin	3.9×10^4	20°C, pH 7.0	Methemoglobin and NO_3^-	(Herold and Shivashankar, 2003)
Catalase-peroxidase (*Mycobacterium tuberculosis*)	1.4×10^5	37°C, pH 7.4	Compound II and $\cdot NO_2$	(Wengenack *et al*., 1999)

(*Continued*)

Table 2 Continued

Protein	k ($M^{-1}s^{-1}$)	Conditions	Products	Reference
Prostaglandin-endoperoxide synthase 1	1.7×10^7	8°C , pH 7	Compound I and NO_2^-	(Trostchansky *et al*., 2007)
Oxygenase domain of inducible NOS[d]	2.2×10^5	pH 7.4	Compound II-like heme and $\cdot NO_2$	(Marechal *et al*., 2007)
Chloroperoxidase[d]	3.8×10^6	23°C, pH 7.1	Compound II and $\cdot NO_2$	(Gebicka and Didik, 2007)
Cytochrome P450 102 (*Bacillus megaterium*)[d]	1×10^6	12°C, pH 6.8	Compound II and $\cdot NO_2$[a]	(Daiber *et al*., 2000)
FeS-clusters				
Mitochondrial [4Fe4S] aconitase	1.1×10^5	25°C, pH 7.6	[3Fe4S]aconitase and $\cdot NO_2$	(Tortora *et al*., 2007)
Zn-thiolate center				
Alcohol dehydrogenase (yeast)	2.6-5.2×10^5	23°C, pH 7.4	Zn release and thiolate oxidation	(Crow *et al*., 1995)
Mn proteins				
Human Mn superoxide dismutase	1×10^5	37°C, pH 7.47	$O = Mn^{IV}$ SOD and $\cdot NO_2$[b]	(Quijano *et al*., 2001)
Cu^{2+} proteins				
Human CuZn superoxide dismutase	1×10^4	37°C, pH 7.5	$Cu^{2+}\cdot OH\cdot SOD + \cdot NO_2$	(Alvarez *et al*., 2004)

[a]Leading to tyrosine 334 nitration (which is not so near the heme; it is an example of intramolecular electron transfer according to the authors) and thiolate oxidation, which is responsible for enzyme inactivation.

[b]Leading to Tyr 34 nitration and dimerization.

[c]RT = room temperature, ND = no appreciable reaction under the experimental conditions employed.

[d]Heme thiolate proteins.

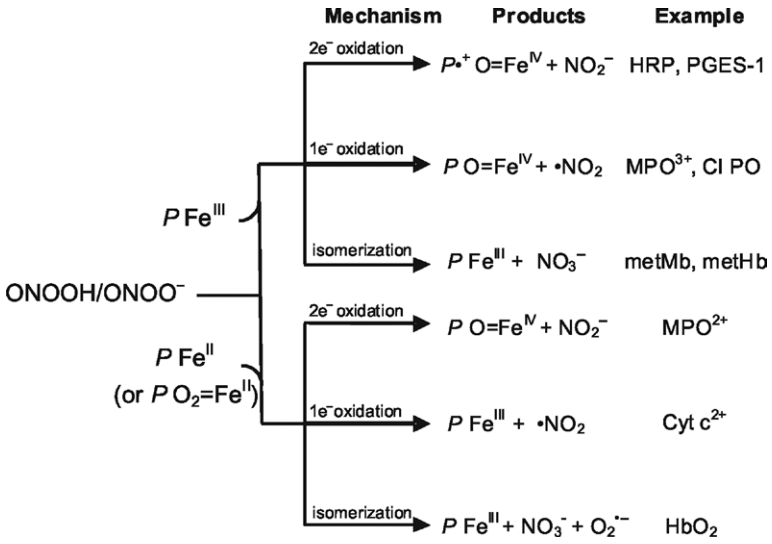

FIGURE 3

Mechanisms of peroxynitrite-mediated heme protein oxidation. Peroxynitrite-mediated two-electron oxidation of Fe^{III}heme proteins, such as horseradish peroxidase (HRP) and prostaglandin endoperoxide H synthase-1 (PGES-1), results in the peroxidase compound I ($P^{\bullet+}$ O = Fe^{IV}) and NO_2^- (Floris *et al.*, 1993; Trostchansky *et al.*, 2007), where $P^{\bullet+}$ is the porphyrin radical cation. Fe^{III}heme protein one-electron oxidation by peroxynitrite, as in ferric myeloperoxidase (MPO^{3+}) and chloroperoxidase (Cl PO), results in peroxidase compound II (P O = Fe^{IV}) and $^{\bullet}NO_2$ (Floris *et al.*, 1993; Gebicka and Didik, 2007). Metmyoglobin (metMb) and methemoglobin (metHb) catalyze peroxynitrite isomerization to NO_3^- (Herold and Shivashankar, 2003). In the case of Fe^{II} heme proteins, peroxynitrite-mediated two-electron oxidation leads to peroxidase compound II and NO_2^-, as for ferrous MPO (MPO^{2+}) (Furtmuller *et al.*, 2005), while in one-electron oxidations Fe^{III} heme protein is formed, as observed in cytochrome c^{2+} (Cyt c^{2+}) (Thomson *et al.*, 1995). Oxyhemoglobin (HbO_2) catalyzes peroxynitrite isomerization to NO_3^-, with simultaneous $O_2^{\bullet-}$ release (Romero *et al.*, 2003).

2004) do not react with peroxynitrite in the ferric state. In the case of the one-electron oxidation of cytochrome c, peroxynitrite reacted with the ferrous but not the ferric form, possibly through an outer sphere electron transfer process (Thomson *et al.*, 1995).

In summary, depending on the transition metal center, the outcome of peroxynitrite reaction can be decomposition to nitrate or nitrite or enhancement of one-electron oxidative processes, sometimes leading to protein modification through amino acidic oxidation and/or nitration.

OXYHEMOGLOBIN

Peroxynitrite directly reacts with oxyhemoglobin (oxyHb, $Hb(Fe^{2+})O_2$) with a rate constant of $2 \times 10^4\ M^{-1}s^{-1}$ at 37 °C and pH 7.4 to yield methemoglobin (metHb, $Hb(Fe^{3+})$) as final product (Denicola *et al.*, 1998; Romero and Radi, 2005). Analysis of the pH profile revealed that peroxynitrous acid (ONOOH) is the reacting species (Denicola *et al.*, 1998). The mechanism of the reaction has been controversial. However, after a complete analysis of the reaction products and stoichiometry under conditions of excess protein over peroxynitrite (as expected to occur *in vivo*), we proposed that peroxynitrite reacts with oxyHb displacing superoxide radical from the heme center and yielding a metHb–peroxynitrite compound. This intermediate can rapidly decompose via peroxo bond homolysis to yield 'caged' ferrylHb and $^{\bullet}NO_2$ that mainly recombine to metHb and nitrate. Only a small fraction of the radicals can diffuse out and are responsible for 'free' ferrylHb formation that, by reaction with nearby amino acids, results in low yields of globin-derived radicals and oxidative modifications (Romero *et al.*, 2003).

The reaction of oxyHb with peroxynitrite in the presence of CO_2 has also been studied (Boccini and Herold, 2004; Minetti *et al.*, 2000). However, inside red blood cells the rate

constant value of the reaction between oxyHb and peroxynitrite and the high concentration of this protein (20 mM heme) determine the k′ for this reaction as 340 s^{-1}, indicating that the reaction of peroxynitrite with CO_2 (k′= 60 s^{-1}) would not be relevant inside these cells. Within red blood cells, kinetic analysis indicates that peroxiredoxin 2 (240 µM) is the main target for peroxynitrite ($k' = 3360\,s^{-1}$ at 25°C) (Manta *et al.*, 2008), but oxyhemoglobin may outcompete peroxiredoxin 2 under conditions of bolus peroxynitrite addition or exposure to very high fluxes of peroxynitrite or other peroxides, which could overcome the capability of Prx reduction by the thioredoxin system (Low *et al.*, 2007). In conclusion, oxyHb reaction with peroxynitrite, in contrast to other hemeproteins, does not promote nitration processes and could contribute as a detoxifying pathway for peroxynitrite in red blood cells, converting it to the inert species nitrate and yielding metHb that could be recovered by the enzymatic metHb-reductase system.

ACONITASES

Aconitases are iron-sulfur proteins which catalyze the reversible isomerization of citrate and isocitrate via *cis*-aconitate. While in mitochondria aconitase is part of the citric acid cycle, in the cytosol aconitase is a trans-regulatory factor that controls iron homeostasis at a post-transcriptional level. Both isoforms (mitochondrial, m-; and cytosolic, c-) contain a [4Fe-4S] prosthetic group in which one of the irons, Feα, is not ligated to a protein residue, and thus can bind to hydroxyl groups of substrates or water (Beinert and Kennedy, 1993). Peroxynitrite reacts with the [4Fe-4S]-cluster of m-aconitase, yielding the inactive [3Fe-4S]-enzyme ($k = 1.1 \times 10^5\,M^{-1}s^{-1}$) (Castro *et al.*, 1994; Tortora *et al.*, 2007). Carbon dioxide enhances peroxynitrite-dependent inactivation, via reaction of $CO_3^{\bullet-}$ with the [4Fe-4S]-cluster ($k = 3 \times 10^8\,M^{-1}s^{-1}$) (Tortora *et al.*, 2007). Because of the capacity of peroxynitrite (and secondarily $CO_3^{\bullet-}$) to perform univalent oxidations, its net reactivity towards aconitase will resemble that of $O_2^{\bullet-}$. The aconitase iron-sulfur cluster has a net oxidation state of +2, with the local positive charge of the exposed Fe_α offering an electrostatic attraction for these anionic oxidants. The transfer of a single electron from the exposed cluster to peroxynitrite (and $CO_3^{\bullet-}$) can destabilize the cluster, causing the loss of Fe_α and the inactivation of the enzyme. Peroxynitrite also nitrates tyrosine residues of m-aconitase (Tyr 151 and Tyr 472 for porcine enzyme) (Han *et al.*, 2005), that are located close to the active site, probably reflecting the site-specific formation of $^{\bullet}NO_2$ following the reaction of peroxynitrite with the active [4Fe-4S] cluster.

The reactivity of peroxynitrite toward m-aconitase *in vivo* is further supported by the fact that proteomic analysis of mitochondria obtained from sepsis, diabetes and animal models of amyotrophic lateral sclerosis, as well as aged rats, reveal that m-aconitase is nitrated (Aulak *et al.*, 2001, 2004; Casoni *et al.*, 2005; Kanski *et al.*, 2005; Turko *et al.*, 2003). Nitrated m-aconitase found *in vivo* represents a footprint of reactive nitrogen species formed during pathology (and probably a signal for protein degradation (Bota and Davies, 2002; Bota *et al.*, 2002) but does not reflect the degree of enzyme inactivation since tyrosine nitration is not involved in the mechanisms of inactivation (Tortora *et al.*, 2007).

Peroxynitrite also reacts with the Fe-S cluster of c-aconitase in different cell culture types, promoting total cluster disruption with the consequent enzyme inactivation, but turning on its iron-responsive activity (Castro *et al.*, 1998; Soum *et al.*, 2003). Also, and depending on peroxynitrite concentration, Cys oxidation of apo c-aconitase could result in an iron-responsive activity decrease because Cys residues are necessary for protein–RNA interactions (Bouton *et al.*, 1997; Soum *et al.*, 2003).

Peroxynitrite reactions with coenzymes

In addition to metal cofactors, other prosthetic groups in proteins have been reported to react with peroxynitrite. For example, peroxynitrite and peroxynitrite-derived radicals react with the dithiol dihydrolipoic acid and its corresponding disulfide, lipoic acid, which

are cofactors of mitochondrial dehydrogenases and are also found free in different tissues at micromolar concentrations when orally supplemented (Trujillo and Radi, 2002). Peroxynitrite inactivates the pyruvate dehydrogenase complex *in vitro*, and tyrosine nitration has been postulated as the main cause of protein inactivation, although the role of lipoamide oxidation in the observed inactivation was not evaluated (Richards *et al.*, 2006).

Tetrahydrobiopterin is also oxidized by peroxynitrite (most probably by peroxynitrite-derived radicals). This has been reported to cause NOS uncoupling leading to increased $O_2^{\bullet -}$ production (Kuzkaya *et al.*, 2003; Sun *et al.*, 2008). This mechanism is considered to be an important contributor in the endothelial dysfunction characteristic of many common clinical disorders.

Folate has also been reported to react with peroxynitrite leading to nitrated derivatives (Nakamura *et al.*, 2002). The fully reduced forms of folate, i.e. tetrahydrofolate and 5-methyltetrahydrofolate, had the most prominent scavenging activity (Rezk *et al.*, 2003). However, kinetic data are not available and it is not known whether the coenzymes react with peroxynitrite itself or with its derived radicals.

Tyrosine nitration and other oxidative modifications

MECHANISMS OF TYROSINE REACTION WITH PEROXYNITRITE

Peroxynitrite does not directly react with tyrosine (Alvarez *et al.*, 1999). However, tyrosine nitration is widely utilized as a marker of peroxynitrite formation in biological milieu (Kooy *et al.*, 1997). Although other routes for tyrosine nitration do exist (Gunther *et al.*, 1997; van der Vliet *et al.*, 1997), peroxynitrite is without any doubt among the main nitrating agents *in vivo* (Radi, 2004). Peroxynitrite-mediated tyrosine nitration (as well as dimerization and in some cases, hydroxylation) is an indirect process mediated by free radicals. Acid-catalyzed peroxynitrite homolysis leads to $^{\bullet}OH$ and $^{\bullet}NO_2$ formation. In the case of $^{\bullet}OH$, transient formation of a radical adduct with tyrosine can lead to tyrosyl radical but also to 3-hydroxytyrosine (dopa), which has been detected as a peroxynitrite-mediated modification of tyrosine under acidic pH, in proteins exposed to peroxynitrite *in vitro* and in cellular systems as well as in a model of *Leishmania amazonensis* infection of a mammalian host (Hensley *et al.*, 1997; Linares *et al.*, 2001; Santos *et al.*, 2000). Moreover, both $^{\bullet}OH$ and $^{\bullet}NO_2$ react with tyrosine residues to form the critical intermediate, the tyrosyl radicals, which in turn recombine with another tyrosyl radical or $^{\bullet}NO_2$ to form 3,3′-dityrosine or 3-nitrotyrosine, respectively (Fig. 4). Protein nitration yields are higher in the presence of CO_2, not only because of the higher radical yields, but mostly because of the more selective action of $CO_3^{\bullet -}$ compared with $^{\bullet}OH$ as tyrosine-oxidizing agent. Metal complexes as well as metal-containing proteins can also cause increased tyrosine nitration and dimerization, as described above for CuZnSOD. The fast reaction between several such metal centers with peroxynitrite can lead to equimolar yields of $^{\bullet}NO_2$ and highly oxidizing species such as ferryl oxo compounds (Fig. 4). Additional mechanisms of tyrosine nitration have been postulated, namely peroxynitrite-dependent metal-bound nitronium-like species for electrophilic non-radical substitution (Ischiropoulos *et al.*, 1992), although experimental evidence for such a process is lacking.

TYROSINE NITRATION BY $^{\bullet}NO$ AND $O_2^{\bullet -}$ FLUXES

The role of peroxynitrite as a mediator of biological nitration has been lately challenged not only by the discovery of other possible pathways of tyrosine nitration in biological systems but also by the observation that, in homogenous systems, the simultaneous production of $^{\bullet}NO$ and $O_2^{\bullet -}$ results in nitration yields much lower than those obtained by the bolus addition of previously synthesized peroxynitrite (Pfeiffer and Mayer, 1998; Pfeiffer *et al.*, 2000). Additionally, when peroxynitrite was formed by fluxes of its precursors $^{\bullet}NO$ and $O_2^{\bullet -}$, the maximum nitration yields were obtained when the rates of precursor formation were nearly identical and decreased in the presence of an excess of either of them, determining bell-shaped nitration

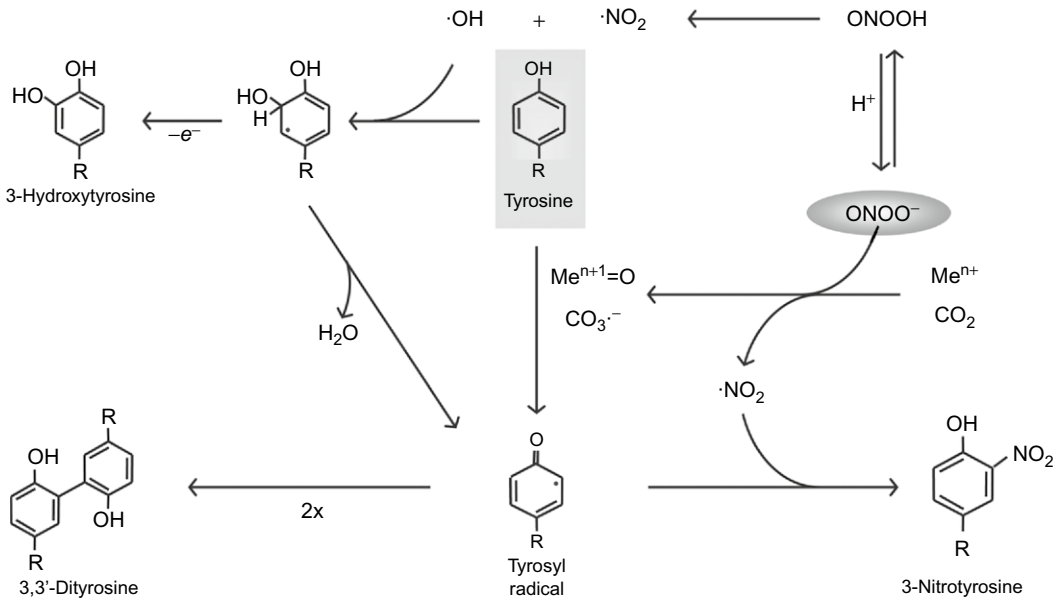

FIGURE 4

Mechanisms of tyrosine modification by peroxynitrite. Peroxynitrite anion ($ONOO^-$) in equilibrium with its conjugated acid peroxynitrous acid (ONOOH) can react with reduced metal centers (Me^{n+}) or carbon dioxide (CO_2) to yield the strong oxidant species oxo-metal complex ($Me^{n+1} = O$) and carbonate radical ($CO_3^{\bullet -}$), respectively, together with nitrogen dioxide radical ($^{\bullet}NO_2$). $Me^{n+1} = O$ and $CO_3^{\bullet -}$ can in turn oxidize tyrosine to tyrosyl radical that in turn combines with $^{\bullet}NO_2$ to yield the post-translational modified amino acid 3-nitrotyrosine. Two tyrosyl radicals can also recombine to yield 3,3′-dityrosine. On the other hand, in the absence of targets, peroxynitrous acid partially (30%) homolyzes to yield $^{\bullet}OH$ and $^{\bullet}NO_2$. The former adds to tyrosine yielding an intermediate adduct that can dehydrate to yield tyrosyl radical or be oxidized to 3-hydroxytyrosine.

profiles. In fact, the modest yields obtained with physiological fluxes of radicals do not contradict *in vivo* data, where 3-nitrotyrosine is not an abundant modification even under inflammatory conditions (Radi, 2004). The difference in nitration yield between bolus peroxynitrite versus fluxes of precursors can be easily understood considering that nitration involves recombination between two radical intermediates ($Tyr^{\bullet}$ and $^{\bullet}NO_2$) present at low concentrations; thus, in spite of the fast kinetic constant for the recombination reaction, the low concentrations of the intermediate reactants allow the direct reaction of tyrosine with $^{\bullet}NO_2$ to yield tyrosyl radical ($k = 3.2 \times 10^5\,M^{-1}s^{-1}$ at pH 7.4; Prutz *et al.*, 1985) to compete with the reaction of $^{\bullet}NO_2$ with tyrosyl radical (Goldstein *et al.*, 2000). As a consequence of $^{\bullet}NO_2$ consumption, tyrosyl radical dimerization outcompetes 3-nitrotyrosine formation. Moreover, the bell-shaped nitration profile obtained in homogenous systems when one of the peroxynitrite precursors is formed at higher rates than the other is owing to reaction of the excess $O_2^{\bullet -}$ with tyrosyl radical or to reaction of excess $^{\bullet}NO$ with $^{\bullet}NO_2$ (Hodges *et al.*, 2000; Sawa *et al.*, 2000), as demonstrated using computer-assisted kinetic simulations. This behavior was hard to reconcile with the solid evidence demonstrating that, in biological systems, peroxynitrite-mediated nitration is associated with an increase in either $^{\bullet}NO$ or $O_2^{\bullet -}$ formation. However, this apparent contradiction between *in vitro* and *in vivo* results can be solved if we consider two key characteristics of biological systems that prevent the accumulation of the peroxynitrite precursors $O_2^{\bullet -}$ and $^{\bullet}NO$: the presence of the enzyme SOD and the facile transmembrane diffusion of $^{\bullet}NO$. SOD is found in different cellular compartments at concentrations in the 1–40 μM range (Chang *et al.*, 1988; Halliwell and Gutteridge, 1999; Quijano *et al.*, 2001), and catalyzes $O_2^{\bullet -}$ dismutation with a rate constant of $2 \times 10^9\,M^{-1}s^{-1}$ (Fielden *et al.*, 1974; Hsu *et al.*, 1996; Klug-Roth *et al.*, 1973), effectively decreasing $O_2^{\bullet -}$ steady state concentrations in the compartment where it is being formed. On the other hand, the lipophilic character of $^{\bullet}NO$ allows it to diffuse outside the cell following its concentration gradient between the places where $^{\bullet}NO$ is formed and consumed, mainly by its reaction with oxyhemoglobin ($k = 8 \times 10^7\,M^{-1}s^{-1}$; Herold *et al.*, 2001).

As a consequence, increases in either $^{\bullet}NO$ or $O_2^{\bullet-}$ fluxes *in vivo* will serve to trap more of the partner radical (and increase peroxynitrite formation) instead of accumulating and interfering with nitration pathways as occurs *in vitro*. Indeed, inclusion of the two 'drain pathways' for excess radicals (the presence of SOD and the facile transmembrane diffusion of $^{\bullet}NO$) in the computer-assisted kinetic model (Quijano *et al.*, 2005) modified the bell-shaped nitration profiles obtained as a function of peroxynitrite precursor flux ratios to a new behavior, where the extent of 3-nitrotyrosine becomes responsive to increases in either $O_2^{\bullet-}$ or $^{\bullet}NO$, in agreement with *in vivo* data.

Another important observation was made in studies of SOD inactivation by fluxes of $^{\bullet}NO$ and $O_2^{\bullet-}$ (Demicheli *et al.*, 2007). These studies clearly show that biologically relevant concentrations of SOD compete with $^{\bullet}NO$, greatly decreasing $O_2^{\bullet-}$ steady state concentration and leading to important increases in $^{\bullet}NO$ steady state that, in turn, better compete with SOD for $O_2^{\bullet-}$; as a consequence, in spite of the dramatic decrease in $O_2^{\bullet-}$ steady state levels, peroxynitrite steady state is only partially affected.

An important dilemma is presented by the fact that glutathione, which is present in cells at mM concentrations, reacts rapidly with $^{\bullet}NO_2$ ($k = 2 \times 10^7\ M^{-1}s^{-1}$, (Ford *et al.*, 2002)) and effectively inhibits nitration mediated by peroxynitrite, $^{\bullet}NO$ plus $O_2^{\bullet-}$ fluxes, or peroxidases in the presence of nitrite and H_2O_2. Therefore, biological tyrosine nitration should predominate in those milieus where glutathione is scarce, such as membranes or extracellular compartments, or under conditions of decreased glutathione concentrations, as occur in pathological conditions associated with oxidative stress (Bharath and Andersen, 2005; Martin *et al.*, 2000). Alternatively, non-$^{\bullet}NO_2$-dependent mechanisms for protein nitration such as oxidation of nitrosotyrosine should be invoked (Demicheli *et al.*, 2007; Gunther *et al.*, 1997). Site-specific nitration, involving the reaction of peroxynitrite within a protein metal center yielding nitrogen dioxide that reacts *in situ* with a tyrosyl radical formed in a nearby residue, is also a possibility to consider.

EFFECTS OF PROTEIN TYROSINE NITRATION

According to most data, protein tyrosine nitration is a relatively selective process, i.e. only a few proteins get nitrated and, within them, only one or a few tyrosine residues are modified. In the case of metalloproteins, selectivity can be easily explained since nitrated tyrosine residues are usually those close to the metal that catalyzes the nitration process, as will be illustrated by the example of MnSOD. In the case of non-metalloproteins, selectivity is harder to explain. It is clearly not related to protein abundance nor the number of tyrosine residues per protein *per se* (Souza *et al.*, 1999). When analyzing nitrated proteins from both *in vitro* experiments and from aged rat tissues, no amino acid consensus sequence or pattern in protein primary structure was apparent (Kanski *et al.*, 2005; Souza *et al.*, 1999). Some common features were recognized that could facilitate specific tyrosine nitration; namely, the presence of acidic residues and of turn-inducing amino acids such as proline and glycine, as well as the absence of cysteine residues in the vicinity of the modified tyrosine (Lin *et al.*, 2005; Souza *et al.*, 1999; Souza *et al.*, 2008b). In the case of cytochrome P450 2B1, Glu149 has been shown to direct the selective nitration of Tyr190 (Lin *et al.*, 2003; Lin *et al.*, 2005). However, a report of endogenous nitrated brain proteins showed the presence of basic amino acids and cysteine residues close to nitration sites (Sacksteder *et al.*, 2006). Moreover, nitrated tyrosine residues are generally located in loops or irregularly folded regions of secondary structure, although the degree of surface accessibility of tyrosine residues does not always allow prediction of which residue gets nitrated in proteins. Therefore, further investigation is clearly required to understand the molecular basis of the observed selectivity of the tyrosine nitration process. Moreover, the distinct mechanisms of protein tyrosine nitration that could take place simultaneously *in vivo*, even when considering non-metalloproteins, could add complexity to the picture.

METAL-CATALYZED TYROSINE NITRATION: THE EXAMPLE OF MnSOD

Manganese-superoxide dismutase (MnSOD) is the SOD isoform found in the mitochondrial matrix of eukaryotes and various prokaryotes. This enzyme plays a crucial role maintaining low steady-states concentrations of $O_2^{\bullet -}$. Peroxynitrite reacts with MnSOD leading to a site-specific nitration of the critical tyrosine 34 and inactivation of the enzyme (MacMillan-Crow *et al.*, 1998; Yamakura *et al.*, 1998). Together with prostacyclin synthase, MnSOD is one of the representative examples where the sole nitration of a specific tyrosine residue produces complete enzyme inactivation *in vivo* (Zou *et al.*, 1999b). The second order rate constants reported for the reaction with human recombinant and *E. coli* MnSOD were 1.0×10^5 and $1.4 \times 10^5\,M^{-1}s^{-1}$ at pH 7.4 and 37°C, respectively. The reaction with the apoenzyme (obtained by removing the manganese ion from the active site) had a second order rate constant $< 10^4\,M^{-1}s^{-1}$, and replacing the manganese by zinc did not promote nitration, supporting the central role of the manganese atom in the process (Quijano *et al.*, 2001). The mechanism of reaction proposed is a direct reaction between peroxynitrite and the metal center of the enzyme, forming $^{\bullet}NO_2$ and the corresponding oxo-manganese complex, that in turn promote site-specific nitration of the critical nearby Tyr34 and inactivation. The enzyme is also capable of promoting nitration of low molecular weight aromatic compounds. Similar inactivation to that observed with authentic peroxynitrite was also obtained when the enzyme was exposed to simultaneous fluxes of $^{\bullet}NO$ and $O_2^{\bullet -}$, even at flux ratios different from one (Demicheli *et al.*, 2007). The role of Tyr34 nitration in enzyme inactivation was unequivocally demonstrated by generating a recombinant MnSOD in which Tyr34 was translationally replaced by 3-nitrotyrosine. The enzyme retained only 3% enzymatic activity compared to the wild type, indicating that MnSOD nitration at this site, without any other modification, results in enzyme inactivation (Neumann *et al.*, 2008).

Nitration and inactivation of MnSOD have been reported to occur in chronic rejection of human renal allografts (MacMillan-Crow *et al.*, 1996), rat models of angiotensin II-induced hypertension (Guo *et al.*, 2003) and aging (van der Loo *et al.*, 2000). Selective antibodies against MnSOD nitrated at Tyr34 were used to detect it in renal medulla from angiotensin II-infused rats (Xu *et al.*, 2006).

UNCATALYZED TYROSINE NITRATION: THE EXAMPLE OF CYTOCHROME c^{3+}

The heme protein cytochrome c, a mitochondrial peripheral inner membrane protein, participates in electron transfer during cellular respiration. More recently, its role in apoptosis has deserved considerable attention (Jiang and Wang, 2004). In this protein, the iron ion is hexacoordinated, with the fifth and sixth coordination positions interacting with His18 and Met80, respectively. Hence, its reactivity towards peroxides is slow, displaying only a weak peroxidatic activity (Radi *et al.*, 1991c). Ferrocytochrome c (Fe^{2+}) reacts with peroxynitrite with a second order rate constant of $1.3 \times 10^4\,M^{-1}s^{-1}$ at pH 7.4 and 25°C, resulting in the oxidation of the heme group to the ferric state (Thomson *et al.*, 1995), while no significant tyrosine nitration was observed (Cassina *et al.*, 2000). When ferricytochrome c (Fe^{3+}) was treated with peroxynitrite, no reaction was detected at the level of the heme, but tyrosine nitration occurred (Cassina *et al.*, 2000). Using low peroxynitrite fluxes residues Tyr97 and Tyr74 of horse ferricytochrome c were preferentially nitrated (Batthyany *et al.*, 2005). Both residues are far from the heme group (14 Å for Tyr97 and 12 Å for Tyr74) and solvent accessible, suggesting a non-heme-catalyzed nitration reaction. Nitration at Tyr74 promoted rupture of the sixth coordination bond of the heme (with Met80) owing to a steric effect of the nitro group which destabilized the mobile Ω-loop of cytochrome c and transmitted it to the iron center via the nearby Tyr67 (Abriata *et al.*, 2008). These new coordination properties of the heme from the mononitrated species explain the observed increase in peroxidatic activity and also the enrichment of nitration of Tyr67 (which is 7 Å from the heme group) in the dinitrated cytochrome c species, now owing to a heme-catalyzed nitration reaction. Moreover, different mononitrated forms of cytochrome c showed a decreased capability for both mitochondrial electron transfer and apoptosome assembly (Cassina *et al.*,

2000; Souza *et al.*, 2008a). Nitrated cytochrome c has been detected in animal models of disease associated with nitroxidative stress, including rat cerebral cortex after oxygen and glucose deprivation (Alonso *et al.*, 2002), renal ischemia/reperfusion (Cruthirds *et al.*, 2003), and obese mice with leptin synthesis defects (Garcia-Ruiz *et al.*, 2006). Nitrocytochrome c is readily translocated from mitochondria to the cytoplasm and nucleus in non-apoptotic cells and may have antioxidant signaling actions (Godoy *et al.*, 2009).

Other protein modifications: carbonyl formation and protein fragmentation

Protein-bound carbonyls have been extensively used as markers of oxidative damage to proteins (He *et al.*, 1999; Stadtman, 2001). Carbonylation can alter protein function or lead to deleterious intermolecular cross-links and aggregates that preclude their degradation by intracellular proteases. Accumulation of carbonylated proteins has been implicated in the etiology and/or progression of several chronic central nervous system disorders (Butterfield *et al.*, 2006; Picklo *et al.*, 2002). Peroxynitrite is one of the different oxidant species that can lead to protein carbonyl formation (either protein bound or as released products) (Headlam and Davies, 2004; Nowak *et al.*, 2006; Szabo *et al.*, 1997). The mechanism of such modification most probably involves peroxynitrite-derived secondary oxidants. Accordingly, products formed when exposing bovine serum albumin to SIN-1-derived fluxes of peroxynitrite showed a similar profile (though at lower yields) to those formed upon exposing to metal ion/H_2O_2 systems, probably reflecting a common mechanism of reaction, mainly involving aliphatic carbon-centered radicals and subsequent alcoxyl radicals and protein fragmentation (Headlam and Davies, 2004).

Intramolecular electron transfer: when final amino acidic modification differs from initial site of reaction

Peroxynitrite-mediated one-electron oxidations of proteins lead to protein radical formation whose precise location depends both on the mechanisms by which it is formed as well as on the stability of the radical species formed at the particular amino acid residue (Bhattacharjee *et al.*, 2007). The initial site of radical formation may differ from the protein radical finally formed, as has been demonstrated for hydrogen peroxide-mediated myoglobin and peroxynitrite-mediated O oxidation (Bhattacharjee *et al.*, 2007; Romero *et al.*, 2003; Witting *et al.*, 2000). During globin oxidation, radicals have been reported to occur mostly on tyrosine (Davies, 1991; Miki *et al.*, 1989) and/or tryptophan (Gunther *et al.*, 1998) residues, in agreement with radical stabilization by resonance delocalization. In model peptides, electron transfer from tyrosyl radical to cysteine residues to form thiyl radicals was reported to favor disulfide formation and to inhibit tyrosine nitration and dimerization (Zhang *et al.*, 2005a), whereas electron transfer from methionine radicals to tyrosine residues increased tyrosine modifications (Zhang *et al.*, 2008).

Multiple amino acidic modifications

In many cases it is difficult to define which of the protein modification/s is/are the responsible for the observed effect on protein function. Separative techniques that allow the isolation of pure isoforms bearing only one modification (Batthyany *et al.*, 2005), as well as genetic engineering techniques including site-directed mutagenesis (Adachi *et al.*, 2004; Neumann *et al.*, 2008), are invaluable tools for pursuing this aim.

Analytical biochemistry techniques have been used to identify specific proteins modified as well as the modification site(s) both *in vitro* and *in vivo*. In this respect, investigation of biological proteomes and protein-translational modifications requires the use of modern and robust mass spectrometry tools usually combined with immunological techniques. However, with few exceptions, available methodologies have had limited success in complex biological samples (reviewed in Ischiropoulos, 2008). Moreover, complementary approaches including pharmacological tools (Szabo *et al.*, 2007) are usually required in order to assign specific protein modifications to peroxynitrite formation in biological systems.

BIOLOGICAL CONSEQUENCES OF PEROXYNITRITE-MEDIATED PROTEIN OXIDATION AND NITRATION

The biological implications of protein modifications mediated by peroxynitrite rely on different possible major effects; namely: (i) direct change in protein function; (ii) protein aggregation and subsequent biological responses; (iii) modulation of protein turnover; (iv) modulation of signaling processes; and (v) induction of immunological responses. These effects are described below.

Loss vs gain of function

Peroxynitrite-mediated protein modifications, including both oxidation and nitration of specific amino acids, could result in altered protein function. In this regard, tyrosine nitration decreases the pK_a of the phenolic hydroxyl of tyrosine from ~ 10 to 7.2–7.5 (Cleighton, 1993), with could have important consequences for protein structure and function (Fig. 5). However, the amount of residues to be nitrated so as to produce a biologically relevant loss of function is a concern. Protein nitration is a relatively widespread *in vivo* modification observed in a large number of proteins, organs and disease conditions. However, its overall yield is typically low. For example, analysis of human bronchoalveolar lavage proteins showed 480 ± 198 and 53 ± 41 µmol of 3-nitrotyrosine per mol of tyrosine residues in asthmatic patients and control

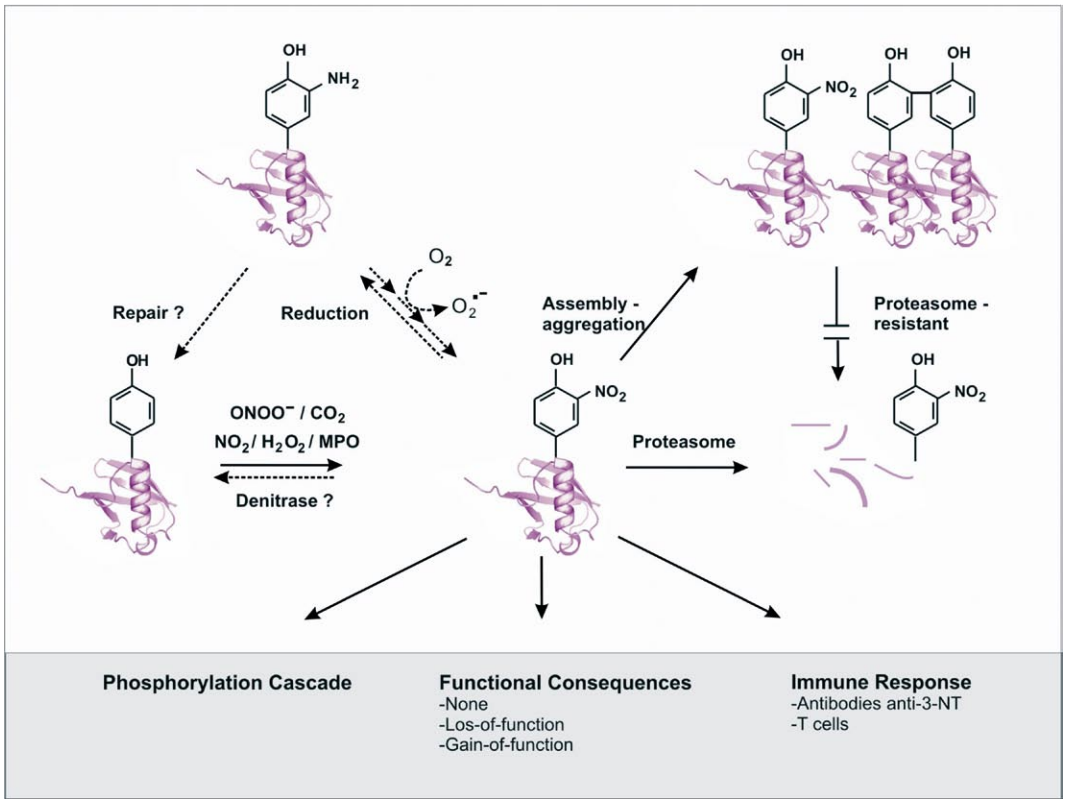

FIGURE 5

Formation and consequences of protein tyrosine nitration. Protein tyrosine nitration has three major effects: it may affect protein function, modulate phosphorylation cascades and induce an immunological response. Usually nitrated proteins are recognized and degraded by the proteasome system. Tyrosine nitration and dimerization may promote assembly of protein filaments or protein aggregates which will also facilitate hydrophobic interactions; these aggregates become poor proteasome substrates, and can accumulate as intra- or extracellular amyloids. Metabolism of nitrated proteins includes the potential reduction by yet-to-be-established biological reductants or the removal of the nitro group by putative denitrase activities; these processes may or may not involve the intermediate formation of 3-aminotyrosine, which in turn may be readily oxidized back to 3-nitrotyrosine (3-NT). Reproduced from Souza *et al.* (2008) with permission from Elsevier.

subjects, respectively (MacPherson *et al.*, 2001); similarly, the average level of protein-bound 3-nitrotyrosine in urine from healthy subjects was reported in the range of 100 µmol 3-nitrotyrosine/mol tyrosine (Chen and Chiu, 2008). Cysteine oxidation to sulfenic acid/disulfide or other peroxynitrite-mediated amino acidic modifications can potentially lead to pronounced changes in protein structure and function. Since peroxynitrite-mediated cysteine oxidation is usually a reversible process, quantitative determinations are difficult to perform.

Taking into account their low abundance, these protein modifications would probably be unable to alter previously existing protein functions. Protein gain of function, on the contrary, could have pronounced biological consequences, even if scarce. The above-described acquisition of an otherwise marginal peroxidatic activity in nitrocytochrome c would be an example. Similarly, oxidation of nerve growth factor by peroxynitrite causes it to acquire an exceptional motor neuron apoptotic activity (Pehar *et al.*, 2006). Other examples where peroxynitrite-mediated protein modification leads to increased activity include the nitration (Tyr92) of rat microsomal glutathione S-transferase (Ji *et al.*, 2006), the S-glutathiolation (Cys674) of sarco/endoplasmic reticulum calcium (Ca^{2+}) ATPase (SERCA) (Adachi *et al.*, 2004), as well as the oxidation to sulfenic acid of Cys298 in aldose reductase (Kaiserova *et al.*, 2008) (Table 1).

Tyrosine oxidation and nitration involved in protein aggregation

Protein aggregation and filament formation have been implicated as key points in the pathogenesis of certain diseases. α-Synuclein can be cited as an example in this field; it is a protein particularly susceptible to the effects of nitrating agents, which in addition to inducing tyrosine nitration, also induce oligomer formation via the oxidation of tyrosine residues to form 3,3′-dityrosine (Souza *et al.*, 2000b). Lewy bodies from Parkinson's, dementia with Lewy bodies and other neurodegenerative diseases are characterized by intracellular α-synuclein filamentous and aggregation inclusions, which were found to be nitrated, cross linked and ubiquitinated (Giasson *et al.*, 2000; Shults, 2006; Spillantini *et al.*, 1997). Monomeric nitrated α-synuclein speeds up the rate of fibril formation and can act as a seed when incubated with the native α-synuclein, inducing the fibrillation process (Hodara *et al.*, 2004). 3,3′-Dityrosine formation in assembled α-synuclein filaments strengthens the filaments to resist denaturing conditions (Souza *et al.*, 2000b), as observed in purified Lewy bodies. In Alzheimer's disease, Tau protein forms intracellular neurofibrillary tangles. It was shown that peroxynitrite induces tyrosine nitration and 3,3′-dityrosine formation as in α-synuclein (Reynolds *et al.*, 2005; Reynolds *et al.*, 2006). In another example, fibrinogen nitration modifies its clotting properties by accelerating thrombin-induced fibrin aggregation and factor XIII cross-linking. A profound distortion of the clot architecture has been observed in the presence of a small percentage of nitrated tyrosine residues, where the clot is transformed into a less elastic structure, more susceptible to embolization (Vadseth *et al.*, 2004). In fact, the level of nitration in circulating fibrinogen is significantly increased under conditions with a high risk of thrombo-embolic episodes, like coronary artery disease and smoking (Parastatidis *et al.*, 2008; Parastatidis *et al.*, 2007). Endogenous fibrinogen nitration is specific for two tyrosine residues, Tyr292 and Tyr422, located in hole-b in the C-terminal side of the β-chain, and their nitration can accelerate fibrin lateral association by changes in the knob-B: hole-b interaction (Parastatidis *et al.*, 2008).

As a general process, we may imagine a scenario where tiny amounts of nitrated or oxidized tyrosine residues from susceptible proteins (i.e. α-synuclein, Tau, fibrinogen) act as a starting point to induce a conformation change in the unmodified protein and promote filamentous aggregates.

Protein degradation

Proteasomes are the main intracellular proteolytic systems involved in altered protein removal, and changes in their activity may contribute to the protein aggregation observed in neurodegenerative diseases (Ding and Keller, 2001; Keller *et al.*, 2002). However, isolated

erythrocyte proteasomes exposed to mild oxidative conditions are stimulated, in line with their role in the cellular antioxidant defense (Strack *et al.*, 1996). Peroxynitrite-mediated protein modifications have been reported to alter protein turnover (Fig. 5). Different peroxynitrite-treated proteins exhibited an enhanced proteolytic susceptibility toward degradation by proteasome (Grune *et al.*, 2001). Peroxynitrite treatment caused an overall activation of the hydrolysis of short peptides by the XYZ 20S proteasome, without changing its caseinolytic activity, whereas, in the case of the immunoproteasome, peroxynitrite-mediated oxidation produced a significant conformational change and concomitant decrease in its enzymatic activity (Amici *et al.*, 2003). Moreover, rat brain Lon protease, an ATP-dependent protease that degrades oxidatively modified aconitase *in vitro* and could play a role in defending against the accumulation of oxidized matrix proteins in mitochondria, is highly susceptible to oxidative inactivation by peroxynitrite. Decline in Lon protease activity preceded electron transport chain dysfunction and was partially reversed by glutathione supplementation of mitochondrial matrix extracts (Stanyer *et al.*, 2008).

Peroxynitrite as a signaling mediator

Alterations in cell oxidative metabolism have long been recognized during differentiation and development. The discovery of specific genes and cell signaling reactions that are affected by oxidants led to the hypothesis that reactive oxygen species serve as cellular messengers in gene regulatory and signal transduction pathways. In fact, in the past few years significant experimental efforts have been devoted to explore the relationships between cellular oxidative processes and the modulation of cell signal transduction, collectively called 'redox signaling' (Allen and Tresini, 2000; Chung *et al.*, 2006; Kramer and Goodyear, 2007). In this regard, the identification of peroxynitrite's ability to nitrate tyrosine residues rapidly focused attention on phosphorylation cascades (Brito *et al.*, 1999; Kong *et al.*, 1996) since 3-nitrotyrosine cannot be phosphorylated. Though this view was initially strongly considered, further investigation showed that peroxynitrite often promotes instead of inhibits phosphorylation signaling, by mechanisms that involve the inhibition of phosphatases via thiol oxidation and the direct activation of different protein tyrosine kinases via nitro-oxidative modification of essential amino acids. Also, novel signaling molecules can be formed by reaction of peroxynitrite with cellular targets, i.e. the formation of nitrolipids with potent PPAR-γ (peroxisome proliferator activating factor γ) agonist activity. In addition, there is considerable cell specificity in terms of response to peroxynitrite. The complexity of the subject is illustrated here with some recent examples.

(a) Endothelial cells respond to proinflammatory stimuli by producing both $^{\bullet}NO$ and $O_2^{\bullet -}$. A concomitant barrier dysfunction can be observed, with consequent edema. Protein phosphatase type 2A (PP2A) mediates dephosphorylation and redistribution of tight junction proteins that can cause paracellular leak in epithelial cell monolayers. Exposure of mouse skeletal muscle microvascular endothelial cell monolayers to lipopolysaccharide and interferon-γ increased permeability, which was attenuated by phosphatase inhibitors or silencing the phosphatase. PP2Ac, the catalytic subunit of PP2A, isolated from these cells, showed increased tyrosine nitration, decreased tyrosine phosphorylation and increased phosphatase activity. Therefore for PP2A, peroxynitrite signaling results in an activation (due to tyrosine nitration) which mediates endothelial barrier dysfunction (Wu and Wilson, 2009). However, other tyrosine phosphatases have been reported to be inactivated by peroxynitrite due to the oxidation of critical cysteine residues (Takakura *et al.*, 1999).

(b) The NF-κB family of transcription factors is an important component of stress-activated cytoprotective signal transduction pathways. Activation mechanisms require phosphorylation, ubiquitination and degradation of the inhibitor protein, IκBα. Ionizing radiation in the therapeutic dose range stimulates NF-κB activity by a mechanism in which IκBα Tyr181 is nitrated as a consequence of constitutive NOS activation,

leading to dissociation of intact IκBα from NF-κB. This mechanism does not appear to require IκBα kinase-dependent phosphorylation or proteolytic degradation of IκBα. Tyrosine 181 is involved in several noncovalent interactions with the p50 subunit of NF-κB, stabilizing the IκBα–NF-κB complex. Evaluation of hydropathic interactions of the IκBα–p50 complex on the basis of the crystal structure of the complex is consistent with nitration disrupting these interactions and dissociating the IκBα–NF-κB complex (Yakovlev *et al.*, 2007). Therefore, tyrosine nitration is an important post-translational regulatory modification for NF-κB activation.

(c) Protein kinase B (Akt) is a serine-threonine protein kinase that plays key roles in integrating cellular responses to growth factors and other extracellular signals. Akt activation also represents an important protective mechanism to limit apoptotic cell death under conditions of oxidative stress. The activation of Akt critically depends on phosphoinositide-3-kinase signaling. In rat cortical neurons, peroxynitrite rapidly elicited Akt-Ser(473) phosphorylation. Inhibition of the phosphoinositide-3-kinase/Akt pathway abolished the ability of peroxynitrite to prevent apoptotic death. Peroxynitrite-stimulated Akt-Ser(473) phosphorylation led to critical cysteine oxidation in phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase (PTEN). Thus, in this neuronal model, peroxynitrite exerts neuroprotection by inhibiting PTEN and hence activating the anti-apoptotic PI3K/Akt pathway (Delgado-Esteban *et al.*, 2007).

In summary, peroxynitrite exhibits typical properties of a signaling molecule *in vitro*, either activating or inhibiting several major signal transduction pathways. Efforts in the near future should focus on consolidating data to confirm the relevance of peroxynitrite as a signaling molecule *in vivo*.

Protein 3-nitrotyrosine as an antigen

Nitration, as well as other oxidant-induced post-translational protein modifications, has been shown to be responsible for eliciting immune responses against autologous and minimally modified proteins. For example, immunization of rabbits or mice with nitrated proteins or peptides resulted in the generation of specific anti-nitrotyrosine antibodies (Beckmann *et al.*, 1994; Brito *et al.*, 1999; Heijnen *et al.*, 2006). Recently, the molecular mechanisms that may govern the immunologic responses to tyrosine-nitrated proteins and peptides have been explored. Transgenic mice that constitutively express exogenous pigeon/moth cytochrome c are tolerant against immunization with the restricted T-cell epitope that encompasses amino acids 88–103 of this protein, but exhibited a robust immune response when Tyr97 was substituted by 3-nitrotyrosine (Birnboim *et al.*, 2003). The same research group proved that the conversion of Tyr4 to 3-nitrotyrosine in the MHC I-restricted epitope of lymphocytic choriomeningitis virus glycoprotein (gp33) completely abrogated recognition by gp33-specific T cells. Conversely, $CD8^+$ T cells specific against nitrated gp33 were readily elicited by immunization with 3-nitrotyrosine-modified gp33 (Hardy *et al.*, 2008). Additionally, monoclonal antibodies against nitrated peptides from hen egg-white lysozyme were elicited in transgenic mice that express the protein as self (Herzog *et al.*, 2005). Notably, an auto-antibody generated by a hybridome isolated from the spleen of BXD2 mice, which spontaneously develop glomerulonephritis and erosive arthritis, reacted against Tyr286-nitrated enolase but not against the native enzyme or against the enzyme nitrated at the Tyr279 residue (Hsu *et al.*, 2006). An adaptative immune response that exacerbated Parkinson's disease in 1-methyl-4-phenyl-1.2.3.6-tetrahydropyridine (MPTP)-intoxicated mice was induced by 3-nitrotyrosine-modified α-synuclein, showing a probable connection between autoimmunity and neurodegeneration (Benner *et al.*, 2008). In the case of patients suffering chronic inflammatory conditions such as rheumatoid arthritis, osteoarthritis and systemic lupus erythematosus, high levels of antibodies against 3-nitrotyrosine were found (Khan and Siddiqui, 2006). The levels of immunoglobulins that recognize 3-nitrotyrosine

were also significantly higher in the plasma of subjects with acute lung injury after a major trauma, as compared with both normal control subjects, and subjects with major trauma that did not develop the lung complication (Thomson *et al.*, 2007). Altogether, these data show that protein nitration can generate the emergence of neo-epitopes, able to trigger immune responses against autologous proteins and profoundly influence immunologic responses in autoimmune as well as inflammatory and degenerative diseases.

CONCLUSIONS

In summary, many biological consequences of peroxynitrite formation are related to post-translational protein modifications. These can be performed by peroxynitrite directly, such as oxidation of fast reacting thiols and transition metal cofactors. In other cases, including protein tyrosine nitration, modifications rely on secondary oxidants. Peroxynitrite-dependent protein modifications have been detected *in vitro* and *in vivo* and can translate into changes in protein function. Their significance in protein turnover and signaling processes is under intense investigation. Moreover, emerging experimental evidence supports the participation of tyrosine nitration in immune responses. The biochemistry of peroxynitrite–protein interactions is associated with physiological and pathophysiological processes; understanding of this field is opening avenues for the treatment of human disorders.

ACKNOWLEDGMENTS

This work was supported by grants from Howard Hughes Medical Institute (HHMI), International Centre of Genetic Engineering and Biotechnology (ICGEB) to RR; Programa de Desarrollo Tecnológico, Ministerio de Educación y Cultura, to BA and MT; Agencia Nacional de Investigación e Innovación-Fondo Clemente Estable to JMS (FCE2007_491), LC (FCE2007_398) and NR (FCE2007_361) and Comisión Sectorial de Investigación Científica (CSIC), Uruguay, to LC, BA and LT. RR is a Howard Hughes International Research Scholar.

REFERENCES

Abbas, K., Breton, J., Drapier, J.C., 2008. The interplay between nitric oxide and peroxiredoxins. Immunobiology 213, 815–822.

Abriata, L.A., Cassina, A., Tortora, V., Marin, M., Souza, J.M., Castro, L., Vila, A.J., Radi, R., 2009. Nitration of solvent-exposed tyrosine 74 on cytochrome c triggers heme iron-methionine 80 bond disruption: Nuclear magnetic resonance and optical spectroscopy studies. J. Biol. Chem. 284, 17–26.

Adachi, T., Weisbrod, R.M., Pimentel, D.R., Ying, J., Sharov, V.S., Schoneich, C., Cohen, R.A., 2004. S-Glutathiolation by peroxynitrite activates SERCA during arterial relaxation by nitric oxide. Nat. Med. 10, 1200–1207.

Allen, R.G., Tresini, M., 2000. Oxidative stress and gene regulation. Free Radic. Biol. Med. 28, 463–499.

Allison, W.S., Benitez, L.V., Johnson, C.L., 1973. The formation of a protein sulfenamide during the inactivation of the acyl phosphatase activity of oxidized glyceraldehyde-3-phosphate dehydrogenase by benzylamine. Biochem. Biophys. Res. Commun. 52, 1403–1409.

Alonso, D., Encinas, J.M., Uttenthal, L.O., Bosca, L., Serrano, J., Fernandez, A.P., Castro-Blanco, S., Santacana, M., Bentura, M.L., Richart, A., Fernandez-Vizarra, P., Rodrigo, J., 2002. Coexistence of translocated cytochrome c and nitrated protein in neurons of the rat cerebral cortex after oxygen and glucose deprivation. Neuroscience 111, 47–56.

Alvarez, B., Rubbo, H., Kirk, M., Barnes, S., Freeman, B.A., Radi, R., 1996. Peroxynitrite-dependent tryptophan nitration. Chem. Res. Toxicol. 9, 390–396.

Alvarez, B., Ferrer-Sueta, G., Freeman, B.A., Radi, R., 1999. Kinetics of peroxynitrite reaction with amino acids and human serum albumin. J. Biol. Chem. 274, 842–848.

Alvarez, B., Demicheli, V., Duran, R., Trujillo, M., Cervenansky, C., Freeman, B.A., Radi, R., 2004. Inactivation of human Cu,Zn superoxide dismutase by peroxynitrite and formation of histidinyl radical. Free Radic. Biol. Med. 37, 813–822.

Amici, M., Lupidi, G., Angeletti, M., Fioretti, E., Eleuteri, A.M., 2003. Peroxynitrite-induced oxidation and its effects on isolated proteasomal systems. Free Radic. Biol. Med. 34, 987–996.

Andres-Mateos, E., Perier, C., Zhang, L., Blanchard-Fillion, B., Greco, T.M., Thomas, B., Ko, H.S., Sasaki, M., Ischiropoulos, H., Przedborski, S., Dawson, T.M., Dawson, V.L., 2007. DJ-1 gene deletion reveals that DJ-1 is an atypical peroxiredoxin-like peroxidase. Proc. Natl. Acad. Sci. USA 104, 14807–14812.

Arora, M., Kumar, A., Kaundal, R.K., Sharma, S.S., 2008. Amelioration of neurological and biochemical deficits by peroxynitrite decomposition catalysts in experimental diabetic neuropathy. Eur. J. Pharmacol. 596, 77–83.

Arteel, G.E., Briviba, K., Sies, H., 1999. Protection against peroxynitrite. FEBS Lett. 445, 226–230.

Aulak, K.S., Miyagi, M., Yan, L., West, K.A., Massillon, D., Crabb, J.W., Stuehr, D.J., 2001. Proteomic method identifies proteins nitrated in vivo during inflammatory challenge. Proc. Natl. Acad. Sci. USA 98, 12056–12061.

Aulak, K.S., Koeck, T., Crabb, J.W., Stuehr, D.J., 2004. Dynamics of protein nitration in cells and mitochondria. Am. J. Physiol. Heart Circ. Physiol. 286, H30–H38.

Barr, S.D., Gedamu, L., 2003. Role of peroxidoxins in Leishmania chagasi survival. Evidence of an enzymatic defense against nitrosative stress. J. Biol. Chem. 278, 10816–10823.

Batthyany, C., Souza, J.M., Duran, R., Cassina, A., Cervenansky, C., Radi, R., 2005. Time course and site(s) of cytochrome c tyrosine nitration by peroxynitrite. Biochemistry 44, 8038–8046.

Beckman, J.S., Beckman, T.W., Chen, J., Marshall, P.A., Freeman, B.A., 1990. Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide. Proc. Natl. Acad. Sci. USA 87, 1620–1624.

Beckman, J.S., Estevez, A.G., Crow, J.P., Barbeito, L., 2001. Superoxide dismutase and the death of motoneurons in ALS. Trends Neurosci. 24, S15–S20.

Beckmann, J.S., Ye, Y.Z., Anderson, P.G., Chen, J., Accavitti, M.A., Tarpey, M.M., White, C.R., 1994. Extensive nitration of protein tyrosines in human atherosclerosis detected by immunohistochemistry. Biol. Chem. Hoppe Seyler 375, 81–88.

Beinert, H., Kennedy, M.C., 1993. Aconitase, a two-faced protein: enzyme and iron regulatory factor. Faseb. J. 7, 1442–1449.

Benner, E.J., Banerjee, R., Reynolds, A.D., Sherman, S., Pisarev, V.M., Tsiperson, V., Nemachek, C., Ciborowski, P., Przedborski, S., Mosley, R.L., Gendelman, H.E., 2008. Nitrated alpha-synuclein immunity accelerates degeneration of nigral dopaminergic neurons. PLoS ONE 3, e1376.

Bharath, S., Andersen, J.K., 2005. Glutathione depletion in a midbrain-derived immortalized dopaminergic cell line results in limited tyrosine nitration of mitochondrial complex I subunits: implications for Parkinson's disease. Antioxid Redox Signal 7, 900–910.

Bhattacharjee, S., Deterding, L.J., Jiang, J., Bonini, M.G., Tomer, K.B., Ramirez, D.C., Mason, R.P., 2007. Electron transfer between a tyrosyl radical and a cysteine residue in hemoproteins: spin trapping analysis. J. Am. Chem. Soc. 129, 13493–13501.

Birnboim, H.C., Lemay, A.M., Lam, D.K., Goldstein, R., Webb, J.R., 2003. Cutting edge: MHC class II-restricted peptides containing the inflammation-associated marker 3-nitrotyrosine evade central tolerance and elicit a robust cell-mediated immune response. J. Immunol. 171, 528–532.

Biteau, B., Labarre, J., Toledano, M.B., 2003. ATP-dependent reduction of cysteine-sulphinic acid by S. cerevisiae sulphiredoxin. Nature 425, 980–984.

Boccini, F., Herold, S., 2004. Mechanistic studies of the oxidation of oxyhemoglobin by peroxynitrite. Biochemistry 43, 16393–16404.

Bonini, M.G., Augusto, O., 2001. Carbon dioxide stimulates the production of thiyl, sulfinyl, and disulfide radical anion from thiol oxidation by peroxynitrite. J. Biol. Chem. 276, 9749–9754.

Bonini, M.G., Radi, R., Ferrer-Sueta, G., Ferreira, A.M., Augusto, O., 1999. Direct EPR detection of the carbonate radical anion produced from peroxynitrite and carbon dioxide. J. Biol. Chem. 274, 10802–10806.

Bota, D.A., Davies, K.J., 2002. Lon protease preferentially degrades oxidized mitochondrial aconitase by an ATP-stimulated mechanism. Nat. Cell Biol. 4, 674–680.

Bota, D.A., Van Remmen, H., Davies, K.J., 2002. Modulation of Lon protease activity and aconitase turnover during aging and oxidative stress. FEBS Lett. 532, 103–106.

Bouton, C., Hirling, H., Drapier, J.C., 1997. Redox modulation of iron regulatory proteins by peroxynitrite. J. Biol. Chem. 272, 19969–19975.

Brito, C., Naviliat, M., Tiscornia, A.C., Vuillier, F., Gualco, G., Dighiero, G., Radi, R., Cayota, A.M., 1999. Peroxynitrite inhibits T lymphocyte activation and proliferation by promoting impairment of tyrosine phosphorylation and peroxynitrite-driven apoptotic death. J. Immunol. 162, 3356–3366.

Briviba, K., Kissner, R., Koppenol, W.H., Sies, H., 1998. Kinetic study of the reaction of glutathione peroxidase with peroxynitrite. Chem. Res. Toxicol. 11, 1398–1401.

Bryk, R., Griffin, P., Nathan, C., 2000. Peroxynitrite reductase activity of bacterial peroxiredoxins. Nature 407, 211–215.

Butterfield, D.A., Perluigi, M., Sultana, R., 2006. Oxidative stress in Alzheimer's disease brain: new insights from redox proteomics. Eur. J. Pharmacol. 545, 39–50.

Carballal, S., Radi, R., Kirk, M.C., Barnes, S., Freeman, B.A., Alvarez, B., 2003. Sulfenic acid formation in human serum albumin by hydrogen peroxide and peroxynitrite. Biochemistry 42, 9906–9914.

Casoni, F., Basso, M., Massignan, T., Gianazza, E., Cheroni, C., Salmona, M., Bendotti, C., Bonetto, V., 2005. Protein nitration in a mouse model of familial amyotrophic lateral sclerosis: possible multifunctional role in the pathogenesis. J. Biol. Chem. 280, 16295–16304.

Cassina, A.M., Hodara, R., Souza, J.M., Thomson, L., Castro, L., Ischiropoulos, H., Freeman, B.A., Radi, R., 2000. Cytochrome c nitration by peroxynitrite. J. Biol. Chem. 275, 21409–21415.

Castro, L., Rodriguez, M., Radi, R., 1994. Aconitase is readily inactivated by peroxynitrite, but not by its precursor, nitric oxide. J. Biol. Chem. 269, 29409–29415.

Castro, L.A., Robalinho, R.L., Cayota, A., Meneghini, R., Radi, R., 1998. Nitric oxide and peroxynitrite-dependent aconitase inactivation and iron-regulatory protein-1 activation in mammalian fibroblasts. Arch Biochem. Biophys. 359, 215–224.

Chang, L.Y., Slot, J.W., Geuze, H.J., Crapo, J.D., 1988. Molecular immunocytochemistry of the CuZn superoxide dismutase in rat hepatocytes. J. Cell Biol. 107, 2169–2179.

Chen, H.J., Chiu, W.L., 2008. Simultaneous detection and quantification of 3-nitrotyrosine and 3-bromotyrosine in human urine by stable isotope dilution liquid chromatography tandem mass spectrometry. Toxicol Lett. 181, 31–39.

Chen, S.N., Hoffman, M.Z., 1973. Rate constants for the reaction of the carbonate radical with compounds of biochemical interest in neutral aqueous solution. Radiat Res. 56, 40–47.

Chung, H.Y., Sung, B., Jung, K.J., Zou, Y., Yu, B.P., 2006. The molecular inflammatory process in aging. Antioxid Redox Signal 8, 572–581.

Claiborne, A., Miller, H., Parsonage, D., Ross, R.P., 1993. Protein-sulfenic acid stabilization and function in enzyme catalysis and gene regulation. Faseb J. 7, 1483–1490.

Creighton, T.E., 1993. Proteins: Structures and Molecular Properties, second ed. Edn. W.H. Freeman and Co, New York.

Cross, A.H., Manning, P.T., Stern, M.K., Misko, T.P., 1997. Evidence for the production of peroxynitrite in inflammatory CNS demyelination. J. Neuroimmunol. 80, 121–130.

Crow, J.P., Beckman, J.S., McCord, J.M., 1995. Sensitivity of the essential zinc-thiolate moiety of yeast alcohol dehydrogenase to hypochlorite and peroxynitrite. Biochemistry 34, 3544–3552.

Crow, J.P., Ye, Y.Z., Strong, M., Kirk, M., Barnes, S., Beckman, J.S., 1997. Superoxide dismutase catalyzes nitration of tyrosines by peroxynitrite in the rod and head domains of neurofilament-L. J. Neurochem. 69, 1945–1953.

Cruthirds, D.L., Novak, L., Akhi, K.M., Sanders, P.W., Thompson, J.A., MacMillan-Crow, L.A., 2003. Mitochondrial targets of oxidative stress during renal ischemia/reperfusion. Arch Biochem. Biophys. 412, 27–33.

Daiber, A., Herold, S., Schoneich, C., Namgaladze, D., Peterson, J.A., Ullrich, V., 2000. Nitration and inactivation of cytochrome P450BM-3 by peroxynitrite. Stopped-flow measurements prove ferryl intermediates. Eur. J. Biochem. 267, 6729–6739.

Davies, M.J., 1991. Identification of a globin free radical in equine myoglobin treated with peroxides. Biochim. Biophys. Acta 1077, 86–90.

Delgado-Esteban, M., Martin-Zanca, D., Andres-Martin, L., Almeida, A., Bolanos, J.P., 2007. Inhibition of PTEN by peroxynitrite activates the phosphoinositide-3-kinase/Akt neuroprotective signaling pathway. J. Neurochem. 102, 194–205.

Demicheli, V., Quijano, C., Alvarez, B., Radi, R., 2007. Inactivation and nitration of human superoxide dismutase (SOD) by fluxes of nitric oxide and superoxide. Free Radic. Biol. Med. 42, 1359–1368.

Denicola, A., Freeman, B.A., Trujillo, M., Radi, R., 1996. Peroxynitrite reaction with carbon dioxide/bicarbonate: kinetics and influence on peroxynitrite-mediated oxidations. Arch. Biochem. Biophys. 333, 49–58.

Denicola, A., Souza, J.M., Radi, R., 1998. Diffusion of peroxynitrite across erythrocyte membranes. Proc. Natl. Acad Sci. U S A 95, 3566–3571.

Diet, A., Abbas, K., Bouton, C., Guillon, B., Tomasello, F., Fourquet, S., Toledano, M.B., Drapier, J.C., 2007. Regulation of peroxiredoxins by nitric oxide in immunostimulated macrophages. J. Biol. Chem. 282, 36199–36205.

Ding, Q., Keller, J.N., 2001. Proteasomes and proteasome inhibition in the central nervous system. Free Radic. Biol. Med. 31, 574–584.

Dubuisson, M., Vander Stricht, D., Clippe, A., Etienne, F., Nauser, T., Kissner, R., Koppenol, W.H., Rees, J.F., Knoops, B., 2004. Human peroxiredoxin 5 is a peroxynitrite reductase. FEBS Lett. 571, 161–165.

Dupret, J.M., Dairou, J., Atmane, N., Rodrigues-Lima, F., 2005. Inactivation of human arylamine N-acetyltransferase 1 by hydrogen peroxide and peroxynitrite. Methods Enzymol. 400, 215–229.

Estevez, A.G., Crow, J.P., Sampson, J.B., Reiter, C., Zhuang, Y., Richardson, G.J., Tarpey, M.M., Barbeito, L., Beckman, J.S., 1999. Induction of nitric oxide-dependent apoptosis in motor neurons by zinc-deficient superoxide dismutase. Science 286, 2498–2500.

Ferrer-Sueta, G., Radi, R., 2009. Chemical biology of peroxynitrite: kinetics, diffusion, and radicals. ACS Chem. Biol. 4, 161–177.

Ferrer-Sueta, G., Batinic-Haberle, I., Spasojevic, I., Fridovich, I., Radi, R., 1999. Catalytic scavenging of peroxynitrite by isomeric Mn(III) N-methylpyridylporphyrins in the presence of reductants. Chem. Res. Toxicol. 12, 442–449.

Fielden, E.M., Roberts, P.B., Bray, R.C., Lowe, D.J., Mautner, G.N., Rotilio, G., Calabrese, L., 1974. Mechanism of action of superoxide dismutase from pulse radiolysis and electron paramagnetic resonance. Evidence that only half the active sites function in catalysis. Biochem. J. 139, 49–60.

Floris, R., Piersma, S.R., Yang, G., Jones, P., Wever, R., 1993. Interaction of myeloperoxidase with peroxynitrite. A comparison with lactoperoxidase, horseradish peroxidase and catalase. Eur. J. Biochem. 215, 767–775.

Fomenko, D.E., Novoselov, S.V., Natarajan, S.K., Lee, B.C., Koc, A., Carlson, B.A., Lee, T.H., Kim, H.Y., Hatfield, D.L., Gladyshev, V.N., 2008. Methionine-R-sulfoxide reductase 1 (MsrB1) knockout Mmice: Roles of MsrB1 in redox regulation and identification of a novel selenoprotein form. J. Biol. Chem..

Ford, E., Hughes, M.N., Wardman, P., 2002. Kinetics of the reactions of nitrogen dioxide with glutathione, cysteine, and uric acid at physiological pH. Free Radic. Biol. Med. 32, 1314–1323.

Fu, Y., Porres, J.M., Lei, X.G., 2001a. Comparative impacts of glutathione peroxidase-1 gene knockout on oxidative stress induced by reactive oxygen and nitrogen species in mouse hepatocytes. Biochem. J. 359, 687–695.

Fu, Y., Sies, H., Lei, X.G., 2001b. Opposite roles of selenium-dependent glutathione peroxidase-1 in superoxide generator diquat- and peroxynitrite-induced apoptosis and signaling. J. Biol. Chem. 276, 43004–43009.

Furtmuller, P.G., Jantschko, W., Zederbauer, M., Schwanninger, M., Jakopitsch, C., Herold, S., Koppenol, W.H., Obinger, C., 2005. Peroxynitrite efficiently mediates the interconversion of redox intermediates of myeloperoxidase. Biochem. Biophys. Res. Commun. 337, 944–954.

Gadelha, F.R., Thomson, L., Fagian, M.M., Costa, A.D., Radi, R., Vercesi, A.E., 1997. Ca2+ -independent permeabilization of the inner mitochondrial membrane by peroxynitrite is mediated by membrane protein thiol cross-linking and lipid peroxidation. Arch. Biochem. Biophys. 345, 243–250.

Galeva, N.A., Esch, S.W., Williams, T.D., Markille, L.M., Squier, T.C., 2005. Rapid method for quantifying the extent of methionine oxidation in intact calmodulin. J. Am. Soc. Mass Spectrom. 16, 1470–1480.

Garcia-Ruiz, I., Rodriguez-Juan, C., Diaz-Sanjuan, T., del Hoyo, P., Colina, F., Munoz-Yague, T., Solis-Herruzo, J.A., 2006. Uric acid and anti-TNF antibody improve mitochondrial dysfunction in ob/ob mice. Hepatology 44, 581–591.

Gatti, R.M., Radi, R., Augusto, O., 1994. Peroxynitrite-mediated oxidation of albumin to the protein-thiyl free radical. FEBS Lett. 348, 287–290.

Gebicka, L., Didik, J., 2003. Mechanism of peroxynitrite interaction with cytochrome c. Acta Biochim. Pol. 50, 815–823.

Gebicka, L., Didik, J., 2007. Kinetic studies of the reaction of heme-thiolate enzyme chloroperoxidase with peroxynitrite. J. Inorg. Biochem. 101, 159–164.

Georgiou, G., Masip, L., 2003. Biochemistry. An overoxidation journey with a return ticket. Science 300, 592–594.

Gerasimov, O.V., Lymar, S.V., 1999. The yield of hydroxyl radical from the decomposition of peroxynitrous acid. Inorg. Chem. 38, 4317–4321.

Giasson, B.I., Duda, J.E., Murray, I.V., Chen, Q., Souza, J.M., Hurtig, H.I., Ischiropoulos, H., Trojanowski, J.Q., Lee, V.M., 2000. Oxidative damage linked to neurodegeneration by selective alpha-synuclein nitration in synucleinopathy lesions. Science 290, 985–989.

Godoy, L.C., Munoz-Pinedo, C., Castro, L., Cardaci, S., Schonhoff, C.M., King, M., Tortora, V., Marin, M., Miao, Q., Jiang, J.F., Kapralov, A., Jemmerson, R., Silkstone, G.G., Patel, J.N., Evans, J.E., Wilson, M.T., Green, D.R., Kagan, V.E., Radi, R., Mannick, J.B., 2009. Disruption of the M80-Fe ligation stimulates the translocation of cytochrome c to the cytoplasm and nucleus in nonapoptotic cells. Proc. Natl. Acad. Sci. USA 106, 2653–2658.

Goldstein, S., Czapski, G., 1995a. Direct and indirect oxidations by peroxynitrite. Inorg. Chem. 34, 4041–4048.

Goldstein, S., Czapski, G., 1995b. The reaction of NO. with O2.- and HO2.: a pulse radiolysis study. Free Radic. Biol. Med. 19, 505–510.

Goldstein, S., Czapski, G., Lind, J., Merényi, G., 2000. Tyrosine nitration by simultaneous generation of $^{\bullet}NO$ and $O_2^{\bullet -}$ under physiological conditions. How the radicals do the job. J. Biol. Chem. 275, 3031–3036.

Goldstein, S., Samuni, A., 2005. Intra- and intermolecular oxidation of oxymyoglobin and oxyhemoglobin induced by hydroxyl and carbonate radicals. Free Radic. Biol. Med. 39, 511–519.

Grune, T., Klotz, L.O., Gieche, J., Rudeck, M., Sies, H., 2001. Protein oxidation and proteolysis by the nonradical oxidants singlet oxygen or peroxynitrite. Free Radic. Biol. Med. 30, 1243–1253.

Gunther, M.R., Hsi, L.C., Curtis, J.F., Gierse, J.K., Marnett, L.J., Eling, T.E., Mason, R.P., 1997. Nitric oxide trapping of the tyrosyl radical of prostaglandin H synthase-2 leads to tyrosine iminoxyl radical and nitrotyrosine formation. J. Biol. Chem. 272, 17086–17090.

Gunther, M.R., Tschirret-Guth, R.A., Witkowska, H.E., Fann, Y.C., Barr, D.P., Ortiz De Montellano, P.R., Mason, R.P., 1998. Site-specific spin trapping of tyrosine radicals in the oxidation of metmyoglobin by hydrogen peroxide. Biochem. J. 330 (Pt 3), 1293–1299.

Guo, W., Adachi, T., Matsui, R., Xu, S., Jiang, B., Zou, M.H., Kirber, M., Lieberthal, W., Cohen, R.A., 2003. Quantitative assessment of tyrosine nitration of manganese superoxide dismutase in angiotensin II-infused rat kidney. Am. J. Physiol. Heart Circ. Physiol. 285, H1396–H1403.

Halliwell, B., Gutteridge, J.M.C., 1999. Antioxidant defences. In: Fr Radicals in Biology and Medicine. Oxford University Press, Oxford, pp. 105–245.

Han, D., Canali, R., Garcia, J., Aguilera, R., Gallaher, T.K., Cadenas, E., 2005. Sites and mechanisms of aconitase inactivation by peroxynitrite: modulation by citrate and glutathione. Biochemistry 44, 11986–11996.

Hardy, L.L., Wick, D.A., Webb, J.R., 2008. Conversion of tyrosine to the inflammation-associated analog 3′-nitrotyrosine at either TCR- or MHC-contact positions can profoundly affect recognition of the MHC class I-restricted epitope of lymphocytic choriomeningitis virus glycoprotein 33 by CD8T cells. J. Immunol. 180, 5956–5962.

Hattori, F., Murayama, N., Noshita, T., Oikawa, S., 2003. Mitochondrial peroxiredoxin-3 protects hippocampal neurons from excitotoxic injury in vivo. J. Neurochem. 86, 860–868.

He, K., Nukada, H., McMorran, P.D., Murphy, M.P., 1999. Protein carbonyl formation and tyrosine nitration as markers of oxidative damage during ischaemia-reperfusion injury to rat sciatic nerve. Neuroscience 94, 909–916.

Headlam, H.A., Davies, M.J., 2004. Markers of protein oxidation: different oxidants give rise to variable yields of bound and released carbonyl products. Free Radic. Biol. Med. 36, 1175–1184.

Heijnen, H.F., van Donselaar, E., Slot, J.W., Fries, D.M., Blachard-Fillion, B., Hodara, R., Lightfoot, R., Polydoro, M., Spielberg, D., Thomson, L., Regan, E.A., Crapo, J., Ischiropoulos, H., 2006. Subcellular localization of tyrosine-nitrated proteins is dictated by reactive oxygen species generating enzymes and by proximity to nitric oxide synthase. Free Radic. Biol. Med. 40, 1903–1913.

Hensley, K., Maidt, M.L., Pye, Q.N., Stewart, C.A., Wack, M., Tabatabaie, T., Floyd, R.A., 1997. Quantitation of protein-bound 3-nitrotyrosine and 3,4-dihydroxyphenylalanine by high-performance liquid chromatography with electrochemical array detection. Anal Biochem. 251, 187–195.

Herold, S., 1998. Kinetic and spectroscopic characterization of an intermediate peroxynitrite complex in the nitrogen monoxide induced oxidation of oxyhemoglobin. FEBS Lett. 439, 85–88.

Herold, S., Shivashankar, K., 2003. Metmyoglobin and methemoglobin catalyze the isomerization of peroxynitrite to nitrate. Biochemistry 42, 14036–14046.

Herold, S., Exner, M., Nauser, T., 2001. Kinetic and mechanistic studies of the NO*-mediated oxidation of oxymyoglobin and oxyhemoglobin. Biochemistry 40, 3385–3395.

Herold, S., Fago, A., Weber, R.E., Dewilde, S., Moens, L., 2004. Reactivity studies of the Fe(III) and Fe(II)NO forms of human neuroglobin reveal a potential role against oxidative stress. J. Biol. Chem. 279, 22841–22847.

Herzog, J., Maekawa, Y., Cirrito, T.P., Illian, B.S., Unanue, E.R., 2005. Activated antigen-presenting cells select and present chemically modified peptides recognized by unique CD4 T cells. Proc. Natl. Acad. Sci. U S A 102, 7928–7933.

Hodara, R., Norris, E.H., Giasson, B.I., Mishizen-Eberz, A.J., Lynch, D.R., Lee, V.M., Ischiropoulos, H., 2004. Functional consequences of alpha-synuclein tyrosine nitration: diminished binding to lipid vesicles and increased fibril formation. J. Biol. Chem. 279, 47746–47753.

Hodges, G.R., Marwaha, J., Paul, T., Ingold, K.U., 2000. A novel procedure for generating both nitric oxide and superoxide in situ from chemical sources at any chosen mole ratio. First application: tyrosine oxidation and a comparison with preformed peroxynitrite. Chem. Res. Toxicol. 13, 1287–1293.

Hofmann, B., Hecht, H.J., Flohe, L., 2002. Peroxiredoxins. Biol. Chem. 383, 347–364.

Hsu, J.L., Hsieh, Y., Tu, C., O'Connor, D., Nick, H.S., Silverman, D.N., 1996. Catalytic properties of human manganese superoxide dismutase. J. Biol. Chem. 271, 17687–17691.

Hsu, H.C., Zhou, T., Kim, H., Barnes, S., Yang, P., Wu, Q., Zhou, J., Freeman, B.A., Luo, M., Mountz, J.D., 2006. Production of a novel class of polyreactive pathogenic autoantibodies in BXD2 mice causes glomerulonephritis and arthritis. Arthritis Rheum. 54, 343–55.

Huang, J., Lin, S.C., Nadershahi, A., Watts, S.W., Sarkar, R., 2008. Role of redox signaling and poly (adenosine diphosphate-ribose) polymerase activation in vascular smooth muscle cell growth inhibition by nitric oxide and peroxynitrite. J. Vasc. Surg. 47, 599–607.

Huie, R.E., Padmaja, S., 1993. The reaction of no with superoxide. Free Radic. Res. Commun. 18, 195–199.

Ikeda, K., Yukihiro Hiraoka, B., Iwai, H., Matsumoto, T., Mineki, R., Taka, H., Takamori, K., Ogawa, H., Yamakura, F., 2007. Detection of 6-nitrotryptophan in proteins by Western blot analysis and its application for peroxynitrite-treated PC12 cells. Nitric Oxide 16, 18–28.

Ischiropoulos, H., 2008. Protein tyrosine nitration-An update. Arch. Biochem. Biophys..

Ischiropoulos, H., Zhu, L., Chen, J., Tsai, M., Martin, J.C., Smith, C.D., Beckman, J.S., 1992. Peroxynitrite-mediated tyrosine nitration catalyzed by superoxide dismutase. Arch. Biochem. Biophys. 298, 431–437.

Jaeger, T., Budde, H., Flohe, L., Menge, U., Singh, M., Trujillo, M., Radi, R., 2004. Multiple thioredoxin-mediated routes to detoxify hydroperoxides in Mycobacterium tuberculosis. Arch. Biochem. Biophys. 423, 182–191.

Ji, Y., Neverova, I., Van Eyk, J.E., Bennett, B.M., 2006. Nitration of tyrosine 92 mediates the activation of rat microsomal glutathione s-transferase by peroxynitrite. J. Biol. Chem. 281, 1986–1991.

Jiang, X., Wang, X., 2004. Cytochrome C-mediated apoptosis. Annu. Rev. Biochem. 73, 87–106.

Jian-Hong, Z., Zhang, X., Roneker, C.A., McClung, J.P., Zhang, S., Thannhauser, T.W., Ripoll, D.R., Sun, Q., Lei, X.G., 2008. Role of copper,zinc-superoxide dismutase in catalyzing nitrotyrosine formation in murine liver. Free Radic. Biol. Med. 45, 611–618.

Jozsef, L., Filep, J.G., 2003. Selenium-containing compounds attenuate peroxynitrite-mediated NF-kappaB and AP-1 activation and interleukin-8 gene and protein expression in human leukocytes. Free Radic. Biol. Med. 35, 1018–1027.

Kaiserova, K., Tang, X.L., Srivastava, S., Bhatnagar, A., 2008. Role of nitric oxide in regulating aldose reductase activation in the ischemic heart. J. Biol. Chem. 283, 9101–9112.

Kanski, J., Behring, A., Pelling, J., Schoneich, C., 2005. Proteomic identification of 3-nitrotyrosine-containing rat cardiac proteins: effects of biological aging. Am. J. Physiol. Heart Circ. Physiol. 288, H371–H381.

Keller, J.N., Gee, J., Ding, Q., 2002. The proteasome in brain aging. Ageing Res. Rev. 1, 279–293.

Khan, F., Siddiqui, A.A., 2006. Prevalence of anti-3-nitrotyrosine antibodies in the joint synovial fluid of patients with rheumatoid arthritis, osteoarthritis and systemic lupus erythematosus. Clin. Chim. Acta 370, 100–107.

Kharitonov, V.G., Sundquist, A.R., Sharma, V.S., 1995. Kinetics of nitrosation of thiols by nitric oxide in the presence of oxygen. J. Biol. Chem. 270, 28158–28164.

Kim, H.Y., Gladyshev, V.N., 2005. Role of structural and functional elements of mouse methionine-S-sulfoxide reductase in its subcellular distribution. Biochemistry 44, 8059–8067.

Kissner, R., Nauser, T., Bugnon, P., Lye, P.G., Koppenol, W.H., 1997. Formation and properties of peroxynitrite as studied by laser flash photolysis, high-pressure stopped-flow technique, and pulse radiolysis. Chem. Res. Toxicol. 10, 1285–1292.

Klug-Roth, D., Fridovich, I., Rabani, J., 1973. Pulse radiolytic investigations of superoxide catalyzed disproportionation. Mechanism for bovine superoxide dismutase. J. Am. Chem. Soc. 95, 2786–2790.

Kong, S.K., Yim, M.B., Stadtman, E.R., Chock, P.B., 1996. Peroxynitrite disables the tyrosine phosphorylation regulatory mechanism: Lymphocyte-specific tyrosine kinase fails to phosphorylate nitrated cdc2(6-20)NH2 peptide. Proc. Natl. Acad. Sci. U S A 93, 3377–3382.

Konorev, E.A., Hogg, N., Kalyanaraman, B., 1998. Rapid and irreversible inhibition of creatine kinase by peroxynitrite. FEBS Lett. 427, 171–174.

Kooy, N.W., Lewis, S.J., Royall, J.A., Ye, Y.Z., Kelly, D.R., Beckman, J.S., 1997. Extensive tyrosine nitration in human myocardial inflammation: evidence for the presence of peroxynitrite. Crit. Care Med. 25, 812–819.

Koppenol, W.H., Moreno, J.J., Pryor, W.A., Ischiropoulos, H., Beckman, J.S., 1992. Peroxynitrite, a cloaked oxidant formed by nitric oxide and superoxide. Chem. Res. Toxicol. 5, 834–842.

Kramer, H.F., Goodyear, L.J., 2007. Exercise, MAPK, and NF-kappaB signaling in skeletal muscle. J. Appl. Physiol. 103, 388–395.

Kuzkaya, N., Weissmann, N., Harrison, D.G., Dikalov, S., 2003. Interactions of peroxynitrite, tetrahydrobiopterin, ascorbic acid, and thiols: implications for uncoupling endothelial nitric-oxide synthase. J. Biol. Chem. 278, 22546–22554.

Landino, L.M., Skreslet, T.E., Alston, J.A., 2004. Cysteine oxidation of tau and microtubule-associated protein-2 by peroxynitrite: modulation of microtubule assembly kinetics by the thioredoxin reductase system. J. Biol. Chem. 279, 35101–35105.

Lin, H.L., Kent, U.M., Zhang, H., Waskell, L., Hollenberg, P.F., 2003. Mutation of tyrosine 190 to alanine eliminates the inactivation of cytochrome P450 2B1 by peroxynitrite. Chem. Res. Toxicol. 16, 129–136.

Lin, H.L., Zhang, H., Waskell, L., Hollenberg, P.F., 2005. The highly conserved Glu149 and Tyr190 residues contribute to peroxynitrite-mediated nitrotyrosine formation and the catalytic activity of cytochrome P450 2B1. Chem. Res. Toxicol. 18, 1203–1210.

Linares, E., Giorgio, S., Mortara, R.A., Santos, C.X., Yamada, A.T., Augusto, O., 2001. Role of peroxynitrite in macrophage microbicidal mechanisms in vivo revealed by protein nitration and hydroxylation. Free Radic. Biol. Med. 30, 1234–1242.

van der Loo, B., Labugger, R., Skepper, J.N., Bachschmid, M., Kilo, J., Powell, J.M., Palacios-Callender, M., Erusalimsky, J.D., Quaschning, T., Malinski, T., Gygi, D., Ullrich, V., Luscher, T.F., 2000. Enhanced peroxynitrite formation is associated with vascular aging. J. Exp. Med. 192, 1731–1744.

Low, F.M., Hampton, M.B., Peskin, A.V., Winterbourn, C.C., 2007. Peroxiredoxin 2 functions as a noncatalytic scavenger of low-level hydrogen peroxide in the erythrocyte. Blood 109, 2611–2617.

Lymar, S.V., Hurst, J.K., 1995. Rapid reaction between peroxynitrite anion and carbon dioxide: implication for biological activity. J. Am. Chem. Soc. 117, 8867–8868.

MacMillan-Crow, L.A., Crow, J.P., Kerby, J.D., Beckman, J.S., Thompson, J.A., 1996. Nitration and inactivation of manganese superoxide dismutase in chronic rejection of human renal allografts. Proc. Natl. Acad. Sci. U S A 93, 11853–11858.

MacMillan-Crow, L.A., Crow, J.P., Thompson, J.A., 1998. Peroxynitrite-mediated inactivation of manganese superoxide dismutase involves nitration and oxidation of critical tyrosine residues. Biochemistry 37, 1613–1622.

MacPherson, J.C., Comhair, S.A., Erzurum, S.C., Klein, D.F., Lipscomb, M.F., Kavuru, M.S., Samoszuk, M.K., Hazen, S.L., 2001. Eosinophils are a major source of nitric oxide-derived oxidants in severe asthma: characterization of pathways available to eosinophils for generating reactive nitrogen species. J. Immunol. 166, 5763–5772.

Madej, E., Folkes, L.K., Wardman, P., Czapski, G., Goldstein, S., 2008. Thiyl radicals react with nitric oxide to form S-nitrosothiols with rate constants near the diffusion-controlled limit. Free Radic. Biol. Med. 44, 2013–2018.

Manta, B., Hugo, M., Ortiz, C., Ferrer-Sueta, G., Trujillo, M., Denicola, A., 2009. The peroxidase and peroxynitrite reductase activity of human erythrocyte peroxiredoxin 2. Arch. Biochem. Biophys. 484, 146–154.

Marechal, A., Mattioli, T.A., Stuehr, D.J., Santolini, J., 2007. Activation of peroxynitrite by inducible nitric-oxide synthase: a direct source of nitrative stress. J. Biol. Chem. 282, 14101–14112.

Marla, S.S., Lee, J., Groves, J.T., 1997. Peroxynitrite rapidly permeates phospholipid membranes. Proc. Natl. Acad. Sci. U S A 94, 14243–14248.

Martin, L.J., Brambrink, A.M., Price, A.C., Kaiser, A., Agnew, D.M., Ichord, R.N., Traystman, R.J., 2000. Neuronal death in newborn striatum after hypoxia-ischemia is necrosis and evolves with oxidative stress. Neurobiol. Dis. 7, 169–191.

Masumoto, H., Sies, H., 1996. The reaction of ebselen with peroxynitrite. Chem. Res. Toxicol. 9, 262–267.

Miki, H., Harada, K., Yamazaki, I., Tamura, M., Watanabe, H., 1989. Electron spin resonance spectrum of Tyr-151 free radical formed in reactions of sperm whale metmyoglobin with ethyl hydroperoxide and potassium irridate. Arch. Biochem. Biophys. 275, 354–362.

Minetti, M., Pietraforte, D., Carbone, V., Salzano, A.M., Scorza, G., Marino, G., 2000. Scavenging of peroxynitrite by oxyhemoglobin and identification of modified globin residues. Biochemistry 39, 6689–6697.

Monteiro, G., Horta, B.B., Pimenta, D.C., Augusto, O., Netto, L.E., 2007. Reduction of 1-Cys peroxiredoxins by ascorbate changes the thiol-specific antioxidant paradigm, revealing another function of vitamin C. Proc. Natl. Acad. Sci. USA 104, 4886–4891.

Moreno, J.J., Pryor, W.A., 1992. Inactivation of alpha 1-proteinase inhibitor by peroxynitrite. Chem. Res. Toxicol. 5, 425–431.

Nakamura, T., Lipton, S.A., 2008. Emerging roles of S-nitrosylation in protein misfolding and neurodegenerative diseases. Antioxid Redox Signal 10, 87–101.

Nakamura, M., Nagayoshi, R., Ijiri, K., Nakashima-Matsushita, N., Takeuchi, T., Matsuyama, T., 2002. Nitration and chlorination of folic acid by peroxynitrite and hypochlorous acid, and the selective binding of 10-nitro-folate to folate receptor beta. Biochem. Biophys. Res. Commun. 297, 1238–1244.

Nelson, K.J., Parsonage, D., Hall, A., Karplus, P.A., Poole, L.B., 2008. Cysteine pKa values for the bacterial peroxiredoxin AhpC. Biochemistry 47, 12860–12868.

Neumann, H., Hazen, J.L., Weinstein, J., Mehl, R.A., Chin, J.W., 2008. Genetically encoding protein oxidative damage. J. Am. Chem. Soc. 130, 4028–4033.

Nickel, C., Trujillo, M., Rahlfs, S., Deponte, M., Radi, R., Becker, K., 2005. Plasmodium falciparum 2-Cys peroxiredoxin reacts with plasmoredoxin and peroxynitrite. Biol. Chem. 386, 1129–1136.

Nowak, P., Saluk-Juszczak, J., Olas, B., Kolodziejczyk, J., Wachowicz, B., 2006. The protective effects of selenoorganic compounds against peroxynitrite-induced changes in plasma proteins and lipids. Cell. Mol. Biol. Lett. 11, 1–11.

Oates, J.C., Christensen, E.F., Reilly, C.M., Self, S.E., Gilkeson, G.S., 1999. Prospective measure of serum 3-nitrotyrosine levels in systemic lupus erythematosus: correlation with disease activity. Proc. Assoc. Am. Physicians 111, 611–621.

Ogusucu, R., Rettori, D., Munhoz, D.C., Netto, L.E., Augusto, O., 2007. Reactions of yeast thioredoxin peroxidases I and II with hydrogen peroxide and peroxynitrite: rate constants by competitive kinetics. Free Radic. Biol. Med. 42, 326–334.

Pacher, P., Beckman, J.S., Liaudet, L., 2007. Nitric oxide and peroxynitrite in health and disease. Physiol. Rev. 87, 315–424.

Parastatidis, I., Thomson, L., Fries, D.M., Moore, R.E., Tohyama, J., Fu, X., Hazen, S. L., Heijnen, H.F., Dennehy, M.K., Liebler, D.C., Rader, D.J., Ischiropoulos, H., 2007. Increased protein nitration burden in the atherosclerotic lesions and plasma of apolipoprotein A-I deficient mice. Circ. Res. 101, 368–376.

Parastatidis, I., Thomson, L., Burke, A., Chernysh, I., Nagaswami, C., Visser, J., Stamer, S., Liebler, D.C., Koliakos, G., Heijnen, H.F., Fitzgerald, G.A., Weisel, J.W., Ischiropoulos, H., 2008. Fibrinogen {beta}-Chain Tyrosine Nitration Is a Prothrombotic Risk Factor. J. Biol. Chem. 283, 33846–33853.

Pearce, L.L., Pitt, B.R., Peterson, J., 1999. The peroxynitrite reductase activity of cytochrome c oxidase involves a two-electron redox reaction at the heme a(3)-Cu(B) site. J. Biol. Chem. 274, 35763–35767.

Pehar, M., Vargas, M.R., Robinson, K.M., Cassina, P., England, P., Beckman, J.S., Alzari, P.M., Barbeito, L., 2006. Peroxynitrite transforms nerve growth factor into an apoptotic factor for motor neurons. Free Radic. Biol. Med. 41, 1632–1644.

Peluffo, G., Radi, R., 2007. Biochemistry of protein tyrosine nitration in cardiovascular pathology. Cardiovasc Res. 75, 291–302.

Perrin, D., Koppenol, W.H., 2000. The quantitative oxidation of methionine to methionine sulfoxide by peroxynitrite. Arch. Biochem. Biophys. 377, 266–272.

Peshenko, I.V., Singh, A.K., Shichi, H., 2001. Bovine eye 1-Cys peroxiredoxin: expression in E. coli and antioxidant properties. J. Ocul. Pharmacol. Ther. 17, 93–99.

Peters, T., 1996. All About Albumin: Biochemistry, Genetics and Medical Applications. Academic Press, San Diego.

Pfeiffer, S., Mayer, B., 1998. Lack of tyrosine nitration by peroxynitrite generated at physiological pH. J. Biol. Chem. 273, 27280–27285.

Pfeiffer, S., Schmidt, K., Mayer, B., 2000. Dityrosine formation outcompetes tyrosine nitration at low steady-state concentrations of peroxynitrite. Implications for tyrosine modification by nitric oxide/superoxide in vivo. J. Biol. Chem. 275, 6346–6352.

Piacenza, L., Peluffo, G., Alvarez, M.N., Kelly, J.M., Wilkinson, S.R., Radi, R., 2008. Peroxiredoxins play a major role in protecting Trypanosoma cruzi against macrophage- and endogenously-derived peroxynitrite. Biochem. J. 410, 359–368.

Picklo, M.J., Montine, T.J., Amarnath, V., Neely, M.D., 2002. Carbonyl toxicology and Alzheimer's disease. Toxicol. Appl. Pharmacol. 184, 187–197.

Pietraforte, D., Minetti, M., 1997. One-electron oxidation pathway of peroxynitrite decomposition in human blood plasma: evidence for the formation of protein tryptophan-centred radicals. Biochem. J. 321 (Pt 3), 743–750.

Prutz, W.A., Monig, H., Butler, J., Land, E.J., 1985. Reactions of nitrogen dioxide in aqueous model systems: oxidation of tyrosine units in peptides and proteins. Arch. Biochem. Biophys. 243, 125–134.

Pryor, W.A., Squadrito, G.L., 1995. The chemistry of peroxynitrite: a product from the reaction of nitric oxide with superoxide. Am. J. Physiol. 268, L699–L722.

Pryor, W.A., Jin, X., Squadrito, G.L., 1994. One- and two-electron oxidations of methionine by peroxynitrite. Proc. Natl. Acad. Sci. U S A 91, 11173–11177.

Quijano, C., Alvarez, B., Gatti, R.M., Augusto, O., Radi, R., 1997. Pathways of peroxynitrite oxidation of thiol groups. Biochem. J. 322 (Pt 1), 167–173.

Quijano, C., Hernandez-Saavedra, D., Castro, L., McCord, J.M., Freeman, B.A., Radi, R., 2001. Reaction of peroxynitrite with Mn-superoxide dismutase. Role of the metal center in decomposition kinetics and nitration. J. Biol. Chem. 276, 11631–11638.

Quijano, C., Romero, N., Radi, R., 2005. Tyrosine nitration by superoxide and nitric oxide fluxes in biological systems: modeling the impact of superoxide dismutase and nitric oxide diffusion. Free Radic. Biol. Med. 39, 728–741.

Rabilloud, T., Heller, M., Gasnier, F., Luche, S., Rey, C., Aebersold, R., Benahmed, M., Louisot, P., Lunardi, J., 2002. Proteomics analysis of cellular response to oxidative stress. Evidence for in vivo overoxidation of peroxiredoxins at their active site. J. Biol. Chem. 277, 19396–19401.

Radi, R., 2004. Nitric oxide, oxidants, and protein tyrosine nitration. Proc. Natl. Acad. Sci. U S A 101, 4003–4008.

Radi, R., Beckman, J.S., Bush, K.M., Freeman, B.A., 1991a. Peroxynitrite-induced membrane lipid peroxidation: the cytotoxic potential of superoxide and nitric oxide. Arch. Biochem. Biophys. 288, 481–487.

Radi, R., Beckman, J.S., Bush, K.M., Freeman, B.A., 1991b. Peroxynitrite oxidation of sulfhydryls. The cytotoxic potential of superoxide and nitric oxide. J. Biol. Chem. 266, 4244–4250.

Radi, R., Thomson, L., Rubbo, H., Prodanov, E., 1991c. Cytochrome c-catalyzed oxidation of organic molecules by hydrogen peroxide. Arch. Biochem. Biophys. 288, 112–117.

Ralat, L.A., Manevich, Y., Fisher, A.B., Colman, R.F., 2006. Direct evidence for the formation of a complex between 1-cysteine peroxiredoxin and glutathione S-transferase pi with activity changes in both enzymes. Biochemistry 45, 360–372.

Rebrin, I., Bregere, C., Kamzalov, S., Gallaher, T.K., Sohal, R.S., 2007. Nitration of tryptophan 372 in succinyl-CoA:3-ketoacid CoA transferase during aging in rat heart mitochondria. Biochemistry 46, 10130–10144.

Reynolds, M.R., Berry, R.W., Binder, L.I., 2005. Site-specific nitration differentially influences tau assembly in vitro. Biochemistry 44, 13997–14009.

Reynolds, M.R., Lukas, T.J., Berry, R.W., Binder, L.I., 2006. Peroxynitrite-mediated tau modifications stabilize preformed filaments and destabilize microtubules through distinct mechanisms. Biochemistry 45, 4314–4326.

Rezk, B.M., Haenen, G.R., van der Vijgh, W.J., Bast, A., 2003. Tetrahydrofolate and 5-methyltetrahydrofolate are folates with high antioxidant activity. Identification of the antioxidant pharmacophore. FEBS Lett. 555, 601–605.

Rhee, S.G., Kang, S.W., Jeong, W., Chang, T.S., Yang, K.S., Woo, H.A., 2005. Intracellular messenger function of hydrogen peroxide and its regulation by peroxiredoxins. Curr. Opin. Cell Biol. 17, 183–189.

Richards, E.M., Rosenthal, R.E., Kristian, T., Fiskum, G., 2006. Postischemic hyperoxia reduces hippocampal pyruvate dehydrogenase activity. Free Radic. Biol. Med. 40, 1960–1970.

Romero, N., Radi, R., 2005. Hemoglobin and red blood cells as tools for studying peroxynitrite biochemistry. Methods Enzymol. 396, 229–245.

Romero, N., Radi, R., Linares, E., Augusto, O., Detweiler, C.D., Mason, R.P., Denicola, A., 2003. Reaction of human hemoglobin with peroxynitrite. Isomerization to nitrate and secondary formation of protein radicals. J. Biol. Chem. 278, 44049–44057.

Sacksteder 2nd, C.A., Qian, W.J., Knyushko, T.V., Wang, H., Chin, M.H., Lacan, G., Melega, W.P., Camp, D.G., Smith, R.D., Smith, D.J., Squier, T.C., Bigelow, D.J., 2006. Endogenously nitrated proteins in mouse brain: links to neurodegenerative disease. Biochemistry 45, 8009–8022.

Sakamoto, A., Tsukamoto, S., Yamamoto, H., Ueda-Hashimoto, M., Takahashi, M., Suzuki, H., Morikawa, H., 2003. Functional complementation in yeast reveals a protective role of chloroplast 2-Cys peroxiredoxin against reactive nitrogen species. Plant J 33, 841–851.

Sandhu, J.K., Robertson, S., Birnboim, H.C., Goldstein, R., 2003. Distribution of protein nitrotyrosine in synovial tissues of patients with rheumatoid arthritis and osteoarthritis. J. Rheumatol. 30, 1173–1181.

Santos, C.X., Bonini, M.G., Augusto, O., 2000. Role of the carbonate radical anion in tyrosine nitration and hydroxylation by peroxynitrite. Arch. Biochem. Biophys. 377, 146–152.

Savvides, S.N., Scheiwein, M., Bohme, C.C., Arteel, G.E., Karplus, P.A., Becker, K., Schirmer, R.H., 2002. Crystal structure of the antioxidant enzyme glutathione reductase inactivated by peroxynitrite. J. Biol. Chem. 277, 2779–2784.

Sawa, T., Akaike, T., Maeda, H., 2000. Tyrosine nitration by peroxynitrite formed from nitric oxide and superoxide generated by xanthine oxidase. J. Biol. Chem. 275, 32467–32474.

Schmidt, K., Pfeiffer, S., Mayer, B., 1998. Reaction of peroxynitrite with HEPES or MOPS results in the formation of nitric oxide donors. Free Radic. Biol. Med. 24, 859–862.

Schmidt, P., Youhnovski, N., Daiber, A., Balan, A., Arsic, M., Bachschmid, M., Przybylski, M., Ullrich, V., 2003. Specific nitration at tyrosine 430 revealed by high resolution mass spectrometry as basis for redox regulation of bovine prostacyclin synthase. J. Biol. Chem. 278, 12813–12819.

Shafirovich, V., Lymar, S.V., 2002. Nitroxyl and its anion in aqueous solutions: spin states, protic equilibria, and reactivities toward oxygen and nitric oxide. Proc. Natl. Acad Sci. USA 99, 7340–7345.

Shults, C.W., 2006. Lewy bodies. Proc. Natl. Acad Sci. USA 103, 1661–1668.

Sies, H., Arteel, G.E., 2000. Interaction of peroxynitrite with selenoproteins and glutathione peroxidase mimics. Free Radic. Biol. Med. 28, 1451–1455.

Smallwood, H.S., Galeva, N.A., Bartlett, R.K., Urbauer, R.J., Williams, T.D., Urbauer, J.L., Squier, T.C., 2003. Selective nitration of Tyr99 in calmodulin as a marker of cellular conditions of oxidative stress. Chem. Res. Toxicol. 16, 95–102.

Soum, E., Brazzolotto, X., Goussias, C., Bouton, C., Moulis, J.M., Mattioli, T.A., Drapier, J.C., 2003. Peroxynitrite and nitric oxide differently target the iron-sulfur cluster and amino acid residues of human iron regulatory protein 1. Biochemistry 42, 7648–7654.

Souza, J.M., Radi, R., 1998. Glyceraldehyde-3-phosphate dehydrogenase inactivation by peroxynitrite. Arch. Biochem. Biophys. 360, 187–194.

Souza, J.M., Daikhin, E., Yudkoff, M., Raman, C.S., Ischiropoulos, H., 1999. Factors determining the selectivity of protein tyrosine nitration. Arch Biochem. Biophys. 371, 169–178.

Souza, J.M., Choi, I., Chen, Q., Weisse, M., Daikhin, E., Yudkoff, M., Obin, M., Ara, J., Horwitz, J., Ischiropoulos, H., 2000a. Proteolytic degradation of tyrosine nitrated proteins. Arch Biochem. Biophys. 380, 360–366.

Souza, J.M., Giasson, B.I., Chen, Q., Lee, V.M., Ischiropoulos, H., 2000b. Dityrosine cross-linking promotes formation of stable alpha -synuclein polymers. Implication of nitrative and oxidative stress in the pathogenesis of neurodegenerative synucleinopathies. J. Biol. Chem. 275, 18344–18349.

Souza, J.M., Castro, L., Cassina, A.M., Batthyany, C., Radi, R., 2008a. Nitrocytochrome c: synthesis, purification, and functional studies. Methods Enzymol. 441, 197–215.

Souza, J.M., Peluffo, G., Radi, R., 2008b. Protein tyrosine nitration--functional alteration or just a biomarker?. Free Radic. Biol. Med. 45, 357–366.

Spillantini, M.G,. Schmidt, M.L., Lee, V.M., Trojanowski, J.Q., Jakes, R., Goedert, M., 1997. Alpha-synuclein in Lewy bodies. Nature 388, 839–840.

St John, G., Brot, N., Ruan, J., Erdjument-Bromage, H., Tempst, P., Weissbach, H., Nathan, C., 2001. Peptide methionine sulfoxide reductase from Escherichia coli and Mycobacterium tuberculosis protects bacteria against oxidative damage from reactive nitrogen intermediates. Proc. Natl. Acad. Sci. USA 98, 9901–9906.

Stadtman, E.R., 2001. Protein oxidation in aging and age-related diseases. Ann. N Y Acad. Sci. 928, 22–38.

Stanyer, L., Jorgensen, W., Hori, O., Clark, J.B., Heales, S.J., 2008. Inactivation of brain mitochondrial Lon protease by peroxynitrite precedes electron transport chain dysfunction. Neurochem Int. 53, 95–101.

Strack, P.R., Waxman, L., Fagan, J.M., 1996. Activation of the multicatalytic endopeptidase by oxidants. Effects on enzyme structure. Biochemistry 35, 7142–7149.

Sumbayev, V.V., Yasinska, I.M., 2006. Peroxynitrite as an alternative donor of oxygen in HIF-1alpha proline hydroxylation under low oxygen availability. Free Radic. Res. 40, 631–635.

Sun, J., Druhan, L.J., Zweier, J.L., 2008. Dose dependent effects of reactive oxygen and nitrogen species on the function of neuronal nitric oxide synthase. Arch Biochem. Biophys. 471, 126–133.

Szabo, C., O'Connor, M., Salzman, A.L., 1997. Endogenously produced peroxynitrite induces the oxidation of mitochondrial and nuclear proteins in immunostimulated macrophages. FEBS Lett. 409, 147–150.

Szabo, C., Ischiropoulos, H., Radi, R., 2007. Peroxynitrite: biochemistry, pathophysiology and development of therapeutics. Nat. Rev. Drug Discov. 6, 662–680.

Takakura, K., Beckman, J.S., MacMillan-Crow, L.A., Crow, J.P., 1999. Rapid and irreversible inactivation of protein tyrosine phosphatases PTP1B, CD45, and LAR by peroxynitrite. Arch Biochem. Biophys. 369, 197–207.

Thomson, L., Trujillo, M., Telleri, R., Radi, R., 1995. Kinetics of cytochrome c2+ oxidation by peroxynitrite: implications for superoxide measurements in nitric oxide-producing biological systems. Arch. Biochem. Biophys. 319, 491–497.

Thomson, L., Christie, J., Vadseth, C., Lanken, P.N., Fu, X., Hazen, S.L., Ischiropoulos, H., 2007. Identification of immunoglobulins that recognize 3-nitrotyrosine in patients with acute lung injury after major trauma. Am. J. Respir Cell Mol. Biol. 36, 152–157.

Tien, M., Berlett, B.S., Levine, R.L., Chock, P.B., Stadtman, E.R., 1999. Peroxynitrite-mediated modification of proteins at physiological carbon dioxide concentration: pH dependence of carbonyl formation, tyrosine nitration, and methionine oxidation. Proc. Natl. Acad. Sci. U S A 96, 7809–7814.

Torreilles, F., Salman-Tabcheh, S., Guerin, M., Torreilles, J., 1999. Neurodegenerative disorders: the role of peroxynitrite. Brain Res. Brain Res. Rev. 30, 153–163.

Tortora, V., Quijano, C., Freeman, B., Radi, R., Castro, L., 2007. Mitochondrial aconitase reaction with nitric oxide, S-nitrosoglutathione, and peroxynitrite: mechanisms and relative contributions to aconitase inactivation. Free Radic Biol. Med. 42, 1075–1088.

Trostchansky, A., O'Donnell, V.B., Goodwin, D.C., Landino, L.M., Marnett, L.J., Radi, R., Rubbo, H., 2007. Interactions between nitric oxide and peroxynitrite during prostaglandin endoperoxide H synthase-1 catalysis: a free radical mechanism of inactivation. Free Radic. Biol. Med. 42, 1029–1038.

Trujillo, M., Radi, R., 2002. Peroxynitrite reaction with the reduced and the oxidized forms of lipoic acid: new insights into the reaction of peroxynitrite with thiols. Arch. Biochem. Biophys. 397, 91–98.

Trujillo, M., Budde, H., Pineyro, M.D., Stehr, M., Robello, C., Flohe, L., Radi, R., 2004. Trypanosoma brucei and Trypanosoma cruzi tryparedoxin peroxidases catalytically detoxify peroxynitrite via oxidation of fast reacting thiols. J. Biol. Chem. 279, 34175–34182.

Trujillo, M., Clippe, A., Manta, B., Ferrer-Sueta, G., Smeets, A., Declercq, J.P., Knoops, B., Radi, R., 2007. Pre-steady state kinetic characterization of human peroxiredoxin 5: taking advantage of Trp84 fluorescence increase upon oxidation. Arch Biochem. Biophys. 467, 95–106.

Trujillo, M., Ferrer-Sueta, G., Radi, R., 2008a. Kinetic studies on peroxynitrite reduction by peroxiredoxins. Methods Enzymol. 441, 173–196.

Trujillo, M., Ferrer-Sueta, G., Radi, R., 2008b. Peroxynitrite detoxification and its biologic implications. Antioxid Redox Signal 10, 1607–1620.

Turell, L., Botti, H., Carballal, S., Ferrer-Sueta, G., Souza, J.M., Duran, R., Freeman, B.A., Radi, R., Alvarez, B., 2008. Reactivity of sulfenic acid in human serum albumin. Biochemistry 47, 358–367.

Turell, L., Botti, H., Carballal, S., Radi, R., Alvarez, B., 2009. Sulfenic acid-A key intermediate in albumin thiol oxidation. J. Chromatogr. B Analyt Technol Biomed Life Sci. Mar 28 [Epub ahead of print].

Turko, I.V., Li, L., Aulak, K.S., Stuehr, D.J., Chang, J.Y., Murad, F., 2003. Protein tyrosine nitration in the mitochondria from diabetic mouse heart. Implications to dysfunctional mitochondria in diabetes. J. Biol. Chem. 278, 33972–33977.

Uppu, R.M., Nossaman, B.D., Greco, A.J., Fokin, A., Murthy, S.N., Fonseca, V.A., Kadowitz, P.J., 2007. Cardiovascular effects of peroxynitrite. Clin. Exp. Pharmacol. Physiol. 34, 933–937.

Vadseth, C., Souza, J.M., Thomson, L., Seagraves, A., Nagaswami, C., Scheiner, T., Torbet, J., Vilaire, G., Bennett, J.S., Murciano, J.C., Muzykantov, V., Penn, M.S., Hazen, S.L., Weisel, J.W., Ischiropoulos, H., 2004. Pro-thrombotic state induced by post-translational modification of fibrinogen by reactive nitrogen species. J. Biol. Chem. 279, 8820–8826.

van der Vliet, A., Eiserich, J.P., Halliwell, B., Cross, C.E., 1997. Formation of reactive nitrogen species during peroxidase-catalyzed oxidation of nitrite. A potential additional mechanism of nitric oxide-dependent toxicity. J. Biol. Chem. 272, 7617–7625.

Wardman, P., 1998. Evaluation of the "radical sink" hypothesis from a chemical-kinetic viewpoint. J. Radianal. Nucl. Chem. 232, 23–27.

Wardman, P., von Sonntag, C., 1995. Kinetic factors that control the fate of thiyl radicals in cells. Methods Enzymol. 251, 31–45.

Wattanapitayakul, S.K., Weinstein, D.M., Holycross, B.J., Bauer, J.A., 2000. Endothelial dysfunction and peroxynitrite formation are early events in angiotensin-induced cardiovascular disorders. Faseb J 14, 271–278.

Wengenack, N.L., Jensen, M.P., Rusnak, F., Stern, M.K., 1999. Mycobacterium tuberculosis KatG is a peroxynitritase. Biochem. Biophys. Res. Commun. 256, 485–487.

Witting, P.K., Douglas, D.J., Mauk, A.G., 2000. Reaction of human myoglobin and H2O2. Involvement of a thiyl radical produced at cysteine 110. J. Biol. Chem. 275, 20391–20398.

Wong, C.M., Zhou, Y., Ng, R.W., Kung Hf, H.F., Jin, D.Y., 2002. Cooperation of yeast peroxiredoxins Tsa1p and Tsa2p in the cellular defense against oxidative and nitrosative stress. J. Biol. Chem. 277, 5385–5394.

Wood, Z.A., Schroder, E., Robin Harris, J., Poole, L.B., 2003. Structure, mechanism and regulation of peroxiredoxins. Trends Biochem. Sci. 28, 32–40.

Wu, F., Wilson, J.X., 2009. Peroxynitrite-dependent activation of protein phosphatase type 2A mediates microvascular endothelial barrier dysfunction. Cardiovasc Res. 81, 38–45.

Xu, S., Ying, J., Jiang, B., Guo, W., Adachi, T., Sharov, V., Lazar, H., Menzoian, J., Knyushko, T.V., Bigelow, D., Schoneich, C., Cohen, R.A., 2006. Detection of sequence-specific tyrosine nitration of manganese SOD and SERCA in cardiovascular disease and aging. Am. J. Physiol. Heart Circ. Physiol. 290, H2220–H2227.

Yakovlev, V.A., Barani, I.J., Rabender, C.S., Black, S.M., Leach, J.K., Graves, P.R., Kellogg, G.E., Mikkelsen, R.B., 2007. Tyrosine nitration of IkappaBalpha: a novel mechanism for NF-kappaB activation. Biochemistry 46, 11671–11683.

Yamakura, F., Ikeda, K., 2006. Modification of tryptophan and tryptophan residues in proteins by reactive nitrogen species. Nitric Oxide 14, 152–161.

Yamakura, F., Taka, H., Fujimura, T., Murayama, K., 1998. Inactivation of human manganese-superoxide dismutase by peroxynitrite is caused by exclusive nitration of tyrosine 34 to 3-nitrotyrosine. J. Biol. Chem. 273, 14085–14089.

Yamakura, F., Matsumoto, T., Ikeda, K., Taka, H., Fujimura, T., Murayama, K., Watanabe, E., Tamaki, M., Imai, T., Takamori, K., 2005. Nitrated and oxidized products of a single tryptophan residue in human Cu,Zn-superoxide dismutase treated with either peroxynitrite-carbon dioxide or myeloperoxidase-hydrogen peroxide-nitrite. J. Biochem 138, 57–69.

Zhang, H., Xu, Y., Joseph, J., Kalyanaraman, B., 2005a. Intramolecular electron transfer between tyrosyl radical and cysteine residue inhibits tyrosine nitration and induces thiyl radical formation in model peptides treated with myeloperoxidase, H2O2, and NO2-: EPR SPIN trapping studies. J. Biol. Chem. 280, 40684–40698.

Zhang, Y.J., Xu, Y.F., Chen, X.Q., Wang, X.C., Wang, J.Z., 2005b. Nitration and oligomerization of tau induced by peroxynitrite inhibit its microtubule-binding activity. FEBS Lett. 579, 2421–2427.

Zhang, H., Zielonka, J., Sikora, A., Joseph, J., Xu, Y., Kalyanaraman, B., 2008. The effect of neighboring methionine residue on tyrosine nitration and oxidation in peptides treated with MPO, H(2)O(2), and NO(2)(-) or peroxynitrite and bicarbonate: Role of intramolecular electron transfer mechanism? Arch. Biochem. Biophys.

Zou, M.H., 2007. Peroxynitrite and protein tyrosine nitration of prostacyclin synthase. Prostaglandins Other Lipid Mediat 82, 119–127.

Zou, M., Yesilkaya, A., Ullrich, V., 1999a. Peroxynitrite inactivates prostacyclin synthase by heme-thiolate-catalyzed tyrosine nitration. Drug Metab Rev. 31, 343–349.

Zou, M.H., Leist, M., Ullrich, V., 1999b. Selective nitration of prostacyclin synthase and defective vasorelaxation in atherosclerotic bovine coronary arteries. Am. J. Pathol. 154, 1359–1365.

Zou, M.H., Cohen, R., Ullrich, V., 2004. Peroxynitrite and vascular endothelial dysfunction in diabetes mellitus. Endothelium 11, 89–97.