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Peroxynitrite formation and function in plants

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ABSTRACT

Peroxynitrite ($ONOO^-$) is a reactive nitrogen species formed when nitric oxide (NO) reacts with the superoxide anion (O_2^-). It was first identified as a mediator of cell death in animals but was later shown to act as a positive regulator of cell signaling, mainly through the posttranslational modification of proteins by tyrosine nitration. In plants, peroxynitrite is not involved in NO-mediated cell death and its physiological function is poorly understood. However, it is emerging as a potential signaling molecule during the induction of defense responses against pathogens and this could be mediated by the selective nitration of tyrosine residues in a small number of proteins. In this review we discuss the general role of tyrosine nitration in plants and evaluate recent evidence suggesting that peroxynitrite is an effector of NO-mediated signaling following pathogen infection.

1. Introduction

Nitric oxide (NO) is a gaseous free radical with a relatively short half-life, which also exists as the nitrosonium cation (NO^+) and nitroxyl anion (NO^-). NO regulates an ever-growing list of biological processes in plants, including growth, development and resistance to stress [1,2]. It has a particularly important role in the hypersensitive response to avirulent pathogens [3]. NO is highly reactive. Oxidative metabolism leads to the formation of numerous derivatives, collectively named reactive nitrogen species (RNS), which include NO_2, N_2O_3, peroxynitrite ($ONOO^-$) and *S*-nitrosoglutathione (GSNO) [4]. RNS can react directly or indirectly with proteins and other molecules in the cell, inducing chemical modifications that lead to changes in structure and function. In this manner, peroxynitrite can act as an effector of NO-dependent signals.

High levels of NO or nitrosothiols (such as GSNO) in the cell cause the *S*-nitrosylation (or transnitrosylation) of cysteine residues in proteins. This involves the addition of an NO group to specific cysteine thiols [5], and can result in the modulation of protein activity (for review see [6–8]). In contrast, high peroxynitrite levels induce a series of reactions targeting lipids, DNA and proteins [9,10] (Fig. 1). Among these reactions, nitration (the addition of a NO_2 group) is one of the most biologically relevant redox mechanisms in animals [9]. Although much less is known about peroxynitrite-mediated nitration in plants, tyrosine nitration in particular is emerging as an important feature of stress responses. This review provides an overview of nitration in plants, focusing on protein tyrosine nitration and its potential role as a signaling regulator during plant defense responses against pathogens.

2. Peroxynitrite-mediated nitration

Peroxynitrite is a strong oxidizing agent, mainly targeting cysteine thiols in proteins [9] and thus inhibiting for instance thiol-containing tyrosine phosphatases, antioxidant enzymes and cysteine proteases. However, peroxynitrite also modifies proteins by the nitration of several amino acids (for review see [11]). Research has focused on the peroxynitrite-mediated modification of tyrosine residues because this forms 3-nitrotyrosine, which is considered a key aspect of peroxynitrite cytotoxicity in animals [12]. Indeed, tyrosine-nitrated proteins become more abundant in all tissues and cell types affected by disease (for review see [9,13]). Protein tyrosine nitration is associated with the production of antigenic epitopes, changes in the catalytic activity of enzymes, altered cytoskeletal organization and impaired signal transduction [14]. Peroxynitrite can also react with tryptophan residues, yielding nitrotryptophan, although the physiological role of this modification, if any, is unclear [11,15]. The proteomic analysis of inflamed neurons has shown that several nitrotryptophan-containing proteins contain functional tryptophan residues that interact with

Abbreviations: APF, aminophenyl fluorescein; GPx, glutathione peroxidase; HR, hypersensitive response; MAPK, mitogen-activated protein kinase; OASA1, O-acetylserine(thiol)lyase A1; PrxIIE, peroxiredoxin IIE; PstAvrB, *Pseudomonas syringae* pv. *tomato* carrying the *AvrB* avirulence gene; RNS, reactive nitrogen species; ROS, reactive oxygen species; SIN-1, 3-morpholinosydnonimine.

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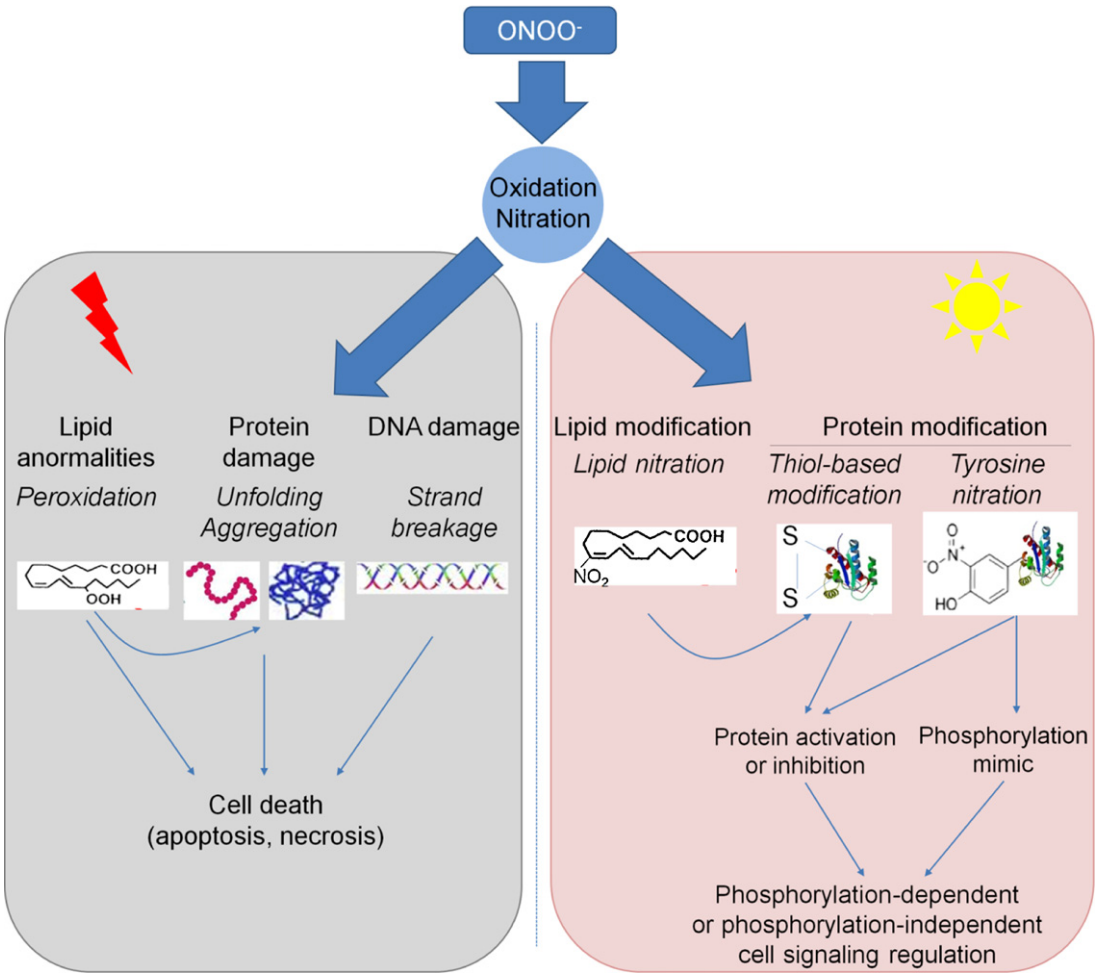

Fig. 1. Dual effects of peroxynitrite on biomolecules. Peroxynitrite triggers a myriad of cytotoxic effects including lipid peroxidation, protein nitration and oxidation, DNA oxidative damage (left panel). If severe enough to overcome cellular antioxidant defenses, the biomolecular injuries initiated by peroxynitrite lead to cell death through apoptosis or necrosis. However, in favorable conditions, the modification of lipids and/or proteins by peroxynitrite participates in the regulation of cell signaling, by interfering for instance with phosphorylation cascades, accounting for a beneficial role of peroxynitrite in modulating cellular response (right panel).

other molecules. These proteins appear to be involved in energy metabolism, protein synthesis and stress responses, and it has been suggested that tryptophan nitration may modulate specific interactions between these proteins and their targets [16].

Nitration can also affect lipids, leading to the formation of various biologically active nitroalken derivates [17]. In animals, nitrated fatty acids act as signaling molecules under normal physiological conditions and in disease, causing changes in protein function via reversible thiol-based modifications [18]. For example, nitroalkylation of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) *in vivo* inhibits the enzyme and causes subcellular translocation [19]. Interestingly, the cytosolic GAPDH from *Arabidopsis thaliana* is also sensitive to NO-mediated *S*-nitrosylation of cysteine residues [20], which suggests that peroxynitrite may mediate the NO-dependent regulation of GAPDH via the formation of nitrated lipids.

Peroxynitrite can also damage DNA, particularly by reacting with guanine to form 8-nitroguanine [21]. This modification promotes DNA cleavage by endonucleases *in vivo*, and the resulting nicks represent a critical aspect of peroxynitrite-mediated cytotoxicity in animals. The formation of 8-nitroguanine activates the nuclear enzyme poly(ADP-ribose) polymerase (PARP), ultimately inducing cell death and tissue inflammation [22]. Moreover, 8-nitroguanine has been shown to act as a pro-oxidant, stimulating superoxide formation by NADPH cytochrome P450 reductases [23]. In contrast to 3-nitrotyrosine, 8-nitroguanine also acts as a mutagen, suggesting a more general role in pathophysiological events [24].

In animals, the frequent association between peroxynitrite-mediated nitration and disease suggests that this modification may be directly involved in disease onset and/or progression. It may trigger or enhance a variety of pro-inflammatory processes, and is a major contributor to both necrosis and apoptosis under severe oxidative stress [25]. However, 3-nitrotyrosine is also present under physiological conditions and tyrosine-nitrated proteins are thought to be involved in normal brain activity and ovulation, which suggests the modification has a physiological role [13]. Accordingly, although tyrosine nitration is often associated with disease, it may also be involved in signal transduction during immune responses and in the regulation of protein metabolism. This new aspect of peroxynitrite-mediated tyrosine nitration is particularly interesting in plants because even high concentrations of peroxynitrite are surprisingly non-toxic to plant cells [26].

3. Protein tyrosine nitration

Posttranslational tyrosine nitration involves the addition of a nitro group at the *ortho* position (with respect to the hydroxyl group) on the aromatic ring [12]. This lowers the pK_a of the phenolic hydroxyl group from 10.1 to 7.2 and adds a bulky adduct as well as a net negative charge at physiological pH. If placed on relevant tyrosine residues, nitration can alter the conformation of a protein

and imposes steric restrictions, thus influencing protein structure and activity [12].

As is the case for other posttranslational modifications, tyrosine nitration appears to occur selectively rather than randomly. Nitration yields are low, with only 1–5 detectable 3-nitrotyrosine residues per 10,000 tyrosines [27,28]. Despite the moderately hydrophilic nature of tyrosine, its relatively high degree of surface exposure (only 15% of tyrosine residues are buried) and the fact that most proteins contain tyrosine (natural abundance 3.2%), only a limited number of proteins are nitration targets and this does not depend on their abundance [29]. For example, human serum albumin is the most abundant plasma protein and it contains 17 tyrosine residues, yet it is not one of the five nitrated proteins identified in plasma samples from patients with acute respiratory distress syndrome [30]. Even within target proteins, only selected tyrosine residues are nitrated. For example, mass spectrometry has shown that only four of the 31 tyrosine residues in human NOS2 are ever subject to nitration [31]. Despite this selectivity, a consensus primary sequence for tyrosine nitration has not been defined, and nitration may thus depend on secondary or higher order structures [29]. The nitration of specific tyrosine residues is promoted by exposing the aromatic ring on the surface of the protein (where tyrosine-containing loop structures are found), by proximity to negatively charged residues and by the absence of nearby cysteine residues [32], although this last criterion is currently subject to debate [29].

Most signaling pathways are characterized by reversible signal transduction mechanisms. Tyrosine nitration has long been considered an irreversible reaction and a general marker for nitrosative stress in animals. However, nitrated proteins can also be detected in healthy animals and plants [33–35], and evidence is emerging that protein nitration may be transient or reversible. In animals, the nitration of tyrosine residues, as phosphorylation, renders proteins more susceptible to proteolysis [36,37]. For example, nitrated CuZn-SOD is degraded by proteasome at nearly twice the rate of the unmodified enzyme [38]. Moreover, some reports propose the existence of a denitrase activity *in vivo* although such an enzyme has not yet been isolated [39–42].

There have been few studies of protein tyrosine nitration in plants, although the proteomic analysis of *A. thaliana* plants challenged with an avirulent pathogen showed that tyrosine-nitrated proteins become more abundant during the infection [34,43]. Despite the absence of a purified denitrase activity and no evidence that nitration induces protein degradation by proteasomes, these studies revealed that the increase in tyrosine-nitrated proteins is a transient phenomenon, suggesting it must be reversible. Eleven target proteins specifically nitrated during the plant hypersensitive disease response have been identified and appear to be involved mainly in photosynthesis, glycolysis and nitrate assimilation [34]. Moreover, nitration was recently proposed as one of the prominent posttranslational modifications in the photosynthetic apparatus [44]. Ongoing studies seek to determine the functional impact of these modifications on the 11 known target proteins. Recently, Alvarez and colleagues [45] demonstrated the inhibition of Arabidopsis O-acetylserine(thiol)lyase A1 (OASA1) by tyrosine nitration. Interestingly, this study showed that OASA1 is much more sensitive to nitration than other members of the same protein family, and mass spectrometry data showed that only one of seven tyrosine residues is nitrated, indicating that the selectivity of tyrosine nitration is conserved among animals and plants.

4. Biological significance of tyrosine nitration

Signaling pathways allow cells to detect environmental changes and generate an appropriate physiological response. In many different animal cell systems, peroxynitrite behaves as a potent modulator of signal transduction pathways by influencing the activity of tyrosine-nitrated proteins [9]. Depending on the protein, tyrosine nitration may have a stimulatory or inhibitory effect, or may even have no effect at all. For example, although mitochondrial MnSOD and glutathione-S-transferase are inhibited by nitration [46], nitrated cytochrome *c* acquires strong peroxidase activity [47]. Similarly, tyrosine nitration results in the activation and translocation of protein kinase C [48].

Tyrosine nitration is thought to interfere with signaling mediated by tyrosine phosphorylation and dephosphorylation, thus potentially affecting a large number of fundamental cellular functions [49,50]. One hypothesis suggests that tyrosine phosphorylation becomes less prevalent as nitration increases, because phosphorylation targets are blocked by the nitro group [37,51]. Accordingly, the nitration of two key tyrosine residues near the C-terminus of the smooth muscle L-type calcium channel prevents phosphorylation by the tyrosine kinase c-Src, thus limiting the influx of calcium [52]. The competition between nitration and phosphorylation for critical tyrosine residues may completely disrupt a complex signal transduction pathway, such as the inhibition of EGFR-mediated apoptosis by nitration of CD95, preventing its phosphorylation by activated EGFR [53]. However, the relationship between nitration and phosphorylation appears to be complex, since the concentration of peroxynitrite can influence whether phosphorylation events are positively or negatively modulated by tyrosine nitration [54]. High peroxynitrite concentrations seem to enhance nitrotyrosine formation and inhibit phosphotyrosine signaling, suggesting direct competition between nitration and phosphorylation of the target tyrosine [55]. However, studies in animal cells have shown that low peroxynitrite concentrations can promote tyrosine phosphorylation [56]. This might reflect the peroxynitrite-dependent activation of protein tyrosine kinases, such as the platelet-derived growth factor receptor (PDGFR), which undergoes tyrosine phosphorylation upon exposure to peroxynitrite through undefined mechanisms [57]. Alternatively, peroxynitrite could inhibit protein tyrosine phosphatases (PTPs). Indeed, the structural similarity between the peroxynitrite and phosphate anions could account for the extreme vulnerability of PTPs to peroxynitrite-mediated inactivation by oxidation of the critical active site cysteine residue [58]. Finally, tyrosine nitration can mimic the effect of phosphorylation by adding a similar negative charge to that produced by phosphorylation, thus activating phosphorylation-mediated signaling pathways [59,60].

In this complex cooperation between nitration and phosphorylation, mitogen-activated protein kinases (MAPKs) represent a key node for the regulation of cell signaling by tyrosine modification in all organisms. MAPK pathways are evolutionary conserved modules in which three kinases act in series, i.e. MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK) and MAPK itself, activated by dual phosphorylation on threonine and tyrosine residues. Depending on the cell type, animal MAPKs can be activated or inhibited by direct tyrosine nitration, as demonstrated for ERK1/2 [61–63] and p38 [64]. Interestingly, the nitration of JNK (a member of the third family of animal MAPKs) does not modify its activity but rather its stability. Indeed, Narang and colleagues [62] reported that tyrosine nitrated JNK is not phosphorylated but remains active and has an extended half-life. Tyrosine nitration can also modulate MAPK activity indirectly by targeting other members of the MAPK module. For example, tyrosine nitration activates the MAPKK MEK1, which in turn activates ERK1/2 [65]. Unexpectedly, we found that NtMEK2 (a MAPKK from tobacco) is inhibited by tyrosine nitration *in vitro*, suggesting that tyrosine nitration could provide a negative feedback mechanism to turn off MAPK signaling in plants (Vandelle and Delledonne, unpublished data). This is particularly relevant in

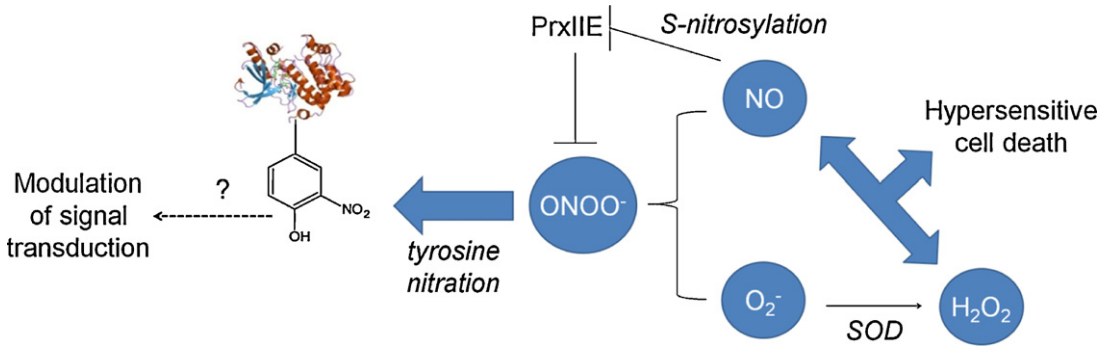

Fig. 2. Peroxynitrite production and metabolism and peroxynitrite-mediated tyrosine nitration during the hypersensitive response. In resistant plants, avirulent pathogens induce a massive concomitant burst of NO and ROS, including superoxide anion and H_2O_2. The fine-tuned balance between NO and H_2O_2 controls the occurrence of the cell death, a key feature of the hypersensitive response. Besides, NO contributes to the mediation of defense signals through the modification of key proteins via *S*-nitrosylation. Among the target proteins, the peroxynitrite-detoxifying activity of Prx IIE is inhibited by this posttranslational modification, contributing to the increase of peroxynitrite level coming from the reaction between NO and O_2^-. In turn, peroxynitrite modifies proteins by nitration of tyrosine residues, which could modulate defense signaling. Abbreviations: ROS, reactive oxygen species; Prx IIE, peroxiredoxin IIE.

the context of plant defense responses, which are determined by the magnitude and duration of MAPK activation [66].

5. Peroxynitrite and tyrosine nitration during the plant hypersensitive response

Peroxynitrite is formed in a diffusion-controlled reaction between stoichiometric amounts of NO and superoxide [67,68]. This takes place mainly at sites where superoxide is produced because the superoxide radical is short-lived and does not diffuse across membranes efficiently, whereas NO is relatively stable and diffusible [69]. The yield of peroxynitrite is limited only by the availability of these precursors [70]. Therefore, in plants, low levels of peroxynitrite are likely to be formed continuously in photosynthesizing chloroplasts, whereas higher levels are likely to be synthesized in response to stress, which induces the production of both NO and reactive oxygen species. The hypersensitive response induced in plants by avirulent pathogens provides this precise scenario, involving the rapid and simultaneous accumulation of NO [71,72] and superoxide [73]. Peroxynitrite is accordingly detected in tobacco cells challenged with the elicitor INF1 [74]. In this study, however, peroxynitrite was detected using the fluorescent probe 3′-(p-aminophenyl)fluorescein (APF), which distinguishes peroxynitrite from NO and O_2^- but cross-reacts with hydroxyl radicals, hypochlorite and peroxy radicals [75,76]. To overcome the problems of data interpretation caused by the lack of APF specificity, Sun and colleagues [77] developed a new BODIPY-type fluorescent probe called Hong-Kong Green-2 (HKGreen-2) which has a similar sensitivity to APF but much greater specificity for peroxynitrite. We developed a photometric assay using this probe and recently demonstrated that infection of *A. thaliana* plants with an avirulent strain of *Pseudomonas syringae* pv. *tomato* (PstAvrB) induces peroxynitrite synthesis approximately 3 h post infection, which peaks after 8 h [78]. This is concomitant with the burst of NO and reactive oxygen species detected in several models in response to avirulent pathogens. For instance, NO and ROS burst peaks 6 h after challenge with an avirulent pathogen in soybean and *A. thaliana* suspension cell cultures [71,79]. In a similar manner, NO production is detected 3–5 h after infiltration of Arabidopsis leaves depending on the avirulence gene [80].

Although peroxynitrite synthesis is not directly controlled by enzymes, it can be detoxified enzymatically to nitrite by some peroxiredoxins in both animals and plants [81]. In plants, this peroxynitrite-detoxifying activity has been demonstrated for two plastid peroxiredoxins, namely PrxIIE and 2-Cys-Prx [43,82]. Therefore, although peroxynitrite synthesis is not regulated in the narrower sense, the steady-state level of this radical is regulated by a network of indirect effectors, which are themselves tightly regulated during the hypersensitive response. The peroxynitrite-detoxifying activity of PrxIIE is inhibited by *S*-nitrosylation a few hours after pathogen infection, indicating that NO induces the accumulation of its own derivative [43] in a timeframe that matches our own analysis of peroxynitrite production during the hypersensitive response [78].

The identification of several proteins subjected to specific tyrosine nitration during the hypersensitive response supports the potential role of peroxynitrite as a mediator of NO signaling during defense responses [34,43]. Nitrotyrosine formation is technically considered a marker of nitrosative stress because it relies on several chemical pathways mediated by peroxynitrite or with nitrite as a substrate in presence of peroxidases and H_2O_2 [9]. However, the coincidence of peroxynitrite accumulation [78] and the increase in tyrosine-nitrated proteins in infected plants [34] strongly suggests that peroxynitrite is likely to be the major nitrating agent in this process. Taken together, these lines of evidence suggest that peroxynitrite formed during the hypersensitive response may mediate NO signaling during this process (Fig. 2).

In animals, peroxynitrite induces cell death and accounts for most of the cytotoxicity attributed to NO. These reactions trigger cellular responses ranging from subtle modulations in cell signaling pathways to overwhelming oxidative injury, committing cells to necrosis or apoptosis [9]. The situation is very different in plants. In soybean cells, the addition of up to 1 mM of peroxynitrite as well as the slower release of peroxynitrite from an NO donor (up to 5 mM SIN1) does not induce cell death [26]. Similarly, the treatment of tobacco cells with urate (a peroxynitrite scavenger) does not influence cell death induced by the elicitor cryptogein, which is partly mediated by NO [83]. Why peroxynitrite is not toxic to plant cells is still unclear. One hypothesis could be the existence of specific detoxifying mechanisms absent in animals. Among them, flavonoids that are known to display a strong anti-oxidant capacity attracted our attention. However, *transparent testa* mutants impaired in flavonoid biosynthesis are not susceptible to peroxynitrite treatment (Vitecek and Delledonne, unpublished data). We are currently investigating other possible mechanisms that could account for peroxynitrite non-toxicity in plants.

Therefore, peroxynitrite is clearly not a death messenger in plants and its function, in particular that related to protein tyrosine nitration, remains to be defined precisely in the context of defense responses against pathogens. It would be interesting to investigate the phosphorylation status of plant proteins in response

to pathogens, as these proteins are likely to be peroxynitrite targets. Indeed, recent studies indicate that tyrosine phosphorylation is more abundant than previously assumed in plant cells and could play an important role in defense signaling [84]. For example, the phosphorylation of one specific tyrosine residue in the receptor kinase BAK1 appears to be critical in regulating its commitment to alternative pathways, including defense responses mediated by flagellin [85].

6. Conclusion

Evaluation of the evidence presented in this article suggests that peroxynitrite could represent a key regulator of signaling between plant cells, particularly in the hypersensitive response against pathogens. However, the presence of several tyrosine-nitrated proteins in uninfected plants [34,35,49] indicates that peroxynitrite signaling is not limited to stress-related pathways and that this radical may be involved in additional physiological roles that have yet to be discovered. The analysis of proteome data to identify proteins that contain nitrotyrosine and other nitro-modified amino acids will provide insight into this phenomenon and help to determine the regulatory networks and signaling pathways in which peroxynitrite plays a significant role.

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References

[1] S. Neill, R. Barros, J. Bright, R. Desikan, J. Hancock, J. Harrison, P. Morris, D. Ribeiro, I. Wilson, Nitric oxide, stomatal closure, and abiotic stress, J. Exp. Bot. 59 (2008) 165–176.
[2] A. Besson-Bard, A. Pugin, D. Wendehenne, New insights into nitric oxide signaling in plants, Annu. Rev. Plant Biol. 59 (2008) 21–39.
[3] M. Delledonne, Y. Xia, R.A. Dixon, C. Lamb, Nitric oxide functions as a signal in plant disease resistance, Nature 394 (1998) 585–588.
[4] C. Nathan, The moving frontier in nitric oxide-dependent signaling, Sci. STKE 2004 (2004) pe52.
[5] J.S. Stamler, D.I. Simon, J.A. Osborne, M.E. Mullins, O. Jaraki, T. Michel, D.J. Singel, J. Loscalzo, S-nitrosylation of proteins with nitric oxide: synthesis and characterization of biologically active compounds, Proc. Natl. Acad. Sci. U. S. A. 89 (1992) 444–448.
[6] M. Leitner, E. Vandelle, F. Gaupels, D. Bellin, M. Delledonne, NO signals in the haze: nitric oxide signaling in plant defense, Curr. Opin. Plant Biol. 12 (2009) 451–458.
[7] C. Lindermayr, J. Durner, S-Nitrosylation in plants: pattern and function, J. Proteomics 73 (2009) 1–9.
[8] S.H. Spoel, Y. Tada, G.J. Loake, Post-translational protein modification as a tool for transcription reprogramming, New Phytol. 186 (2010) 333–339.
[9] P. Pacher, J.S. Beckman, L. Liaudet, Nitric oxide and peroxynitrite in health and disease, Physiol. Rev. 87 (2007) 315–424.
[10] C. Szabo, H. Ischiropoulos, R. Radi, Peroxynitrite: biochemistry, pathophysiology and development of therapeutics, Nat. Rev. 6 (2007) 662–680.
[11] B Alvarez, R. Radi, Peroxynitrite reactivity with amino acids and proteins, Amino Acids 25 (2003) 295–311.
[12] R. Radi, Nitric oxide, oxidants, and protein tyrosine nitration, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 4003–4008.
[13] S.A. Greenacre, H. Ischiropoulos, Tyrosine nitration: localisation, quantification, consequences for protein function and signal transduction, Free Radic. Res. 34 (2001) 541–581.
[14] F.J. Schopfer, P.R. Baker, B.A. Freeman, NO-dependent protein nitration: a cell signaling event or an oxidative inflamatory response? Trends Biochem. Sci. 28 (2003) 646–654.
[15] F. Yamakura, K. Ikeda, Modification of tryptophan and tryptophan residues in proteins by reactive nitrogen species, Nitric Oxide 14 (2006) 152–161.
[16] K. Ikeda, B. Yukihiro Hiraoka, H. Iwai, T. Matsumoto, R. Mineki, H. Taka, K. Takamori, H. Ogawa, F. Yamakura, Detection of 6-nitrotryptophan in proteins by Western blot analysis and its application for peroxynitrite-treated PC12 cells, Nitric Oxide 6 (2007) 18–28.
[17] P.R. Baker, F.J. Schopfer, S. Sweeney, B.A. Freeman, Red cell membrane and plasma linoleic acid nitration products: synthesis, clinical identification, and quantitation, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 11577–11582.
[18] H. Rubbo, R. Radi, Protein and lipid nitration: role in redox signaling and injury, Biochem. Biophys. Acta 1780 (2008) 1318–1324.
[19] C. Batthyany, F.J. Schopfer, P.R. Baker, R. Durán, L.M. Baker, Y. Huang, C. Cerveñansky, B.P. Branchaud, B.A. Freeman, Reversible post-translational modification of proteins by nitrated fatty acids in vivo, J. Biol. Chem. 281 (2006) 20450–20463.
[20] S. Holtgrefe, J. Gohlke, J. Starmann, S. Druce, S. Klocke, B. Altmann, J. Wojtera, C. Lindermayr, R. Scheibe, Regulation of plant cytosolic glyceraldehyde 3-phosphate dehydrogenase isoforms by thiol modifications, Physiol. Plant 133 (2008) 211–228.
[21] J.C. Niles, J.S. Wishnok, S.R. Tannenbau, Peroxynitrite-induced oxidation and nitration products of guanine 8-oxoguanine: structures and mechanisms of product formation, Nitric Oxide 14 (2006) 109–121.
[22] C. Szabo, B. Zingarelli, M. O'Connor, A.L. Salzman, DNA strand breakage, activation of poly (ADP-ribose) synthetase, cellular energy depletion are involved in the cytotoxicity of macrophages and smooth muscle cells exposed to peroxynitrite, Proc. Natl. Acad. Sci. U. S. A. 93 (2003) 1753–1758.
[23] T. Sawa, T. Akaike, K. Ichimori, T. Akuta, K. Kaneko, H. Nakayama, D.J. Stuehr, H. Maeda, Superoxide generation mediated by 8-nitroguanosine, a highly redox-active nucleic acid derivative, Biochem. Biophys. Res. Commun. 311 (2003) 300–306.
[24] T. Akuta, M.H. Zaki, J. Yoshitake, T. Okamoto, T. Akaike, Nitrative stress through formation of 8-nitroguanosine: insights into microbial pathogenesis, Nitric Oxide 14 (2006) 101–108.
[25] L. Liaudet, G. Vassalli, P. Pacher, Role of peroxynitrite in the redox regulation of cell signal transduction pathways, Front Biosci. 14 (2009) 4809–4814.
[26] M. Delledonne, J. Zeier, A. Marocco, C. Lamb, Signal interactions between nitric oxide and reactive oxygen intermediates in the plant hypersensitive disease resistance response, Proc. Natl. Acad. Sci. U. S. A. 98 (2001) 13454–13459.
[27] M.L. Brennan, W. Wu, X. Fu, Z. Shen, W. Song, H. Frost, C. Vadseth, L. Narine, E. Lenkiewicz, M.T. Borchers, A.J. Lusis, J.J. Lee, N.A. Lee, H.M. Abu-Soud, H. Ischiropoulos, S.L. Hazen, A tale of two controversies: defining both the role of peroxidases in nitrotyrosine formation in vivo using eosinophil peroxidase and myeloperoxidase-deficient mice, and the nature of peroxidase-generated reactive nitrogen species, J. Biol. Chem. 277 (2002) 17415–17427.
[28] M. Aslan, T.M. Ryan, T.M. Townes, L. Coward, M.C. Kirk, S. Barnes, C.B. Alexander, S.S. Rosenfeld, B.A. Freeman, Nitric oxide-dependent generation of reactive species in sickle cell disease. Actin tyrosine induces defective cytoskeletal polymerization, J. Biol. Chem. 278 (2003) 4194–4204.
[29] A.S. Bayden, V.A. Yakovlev, P.R. Graves, R.B. Mikkelsen, G.W. Kellogg, Factors influencing protein tyrosine nitration-structure-based predictive models, Free Radic. Biol. Med. 50 (2011) 749–762.
[30] M.D. Gole, J.M. Souza, I. Choi, C. Hertkorn, S. Malcolm, R.F. Foust III, B. Finkel, P.N. Lanken, H. Ischiropoulos, Plasma proteins modified by tyrosine nitration in acute respiratory distress syndrome, Am. J. Physiol. Lung Cell. Mol. Physiol. 278 (2000) L961–L967.
[31] S. Lanone, P. Manivet, J. Callebert, J.M. Launay, D. Payen, M. Aubier, J. Boczkowski, A. Mebazaa, Inducible nitric oxide synthase (NOS2) expressed in septic patients is nitrated on selected tyrosine residues: implications for enzymic activity, Biochem. J. 366 (2002) 399–404.
[32] J.M. Souza, E. Daikhin, M. Yudkoff, C.S. Raman, H. Ischiropoulos, Factors determining the selectivity of protein tyrosine nitration, Arch. Biochem. Biophys. 371 (1999) 169–178.
[33] Y. Sekar, T. Chul Moon, C.M. Slupsky, A.D. Befus, Protein tyrosine nitration of aldolase in mast cells: a plausible pathway in nitric oxide-mediated regulation of mast cell function, J. Immunol. 185 (2010) 578–587.
[34] D. Cecconi, S. Orzetti, E. Vandelle, S. Rinalducci, L. Zolla, M. Delledonne, Protein nitration during defense response in Arabidopsis thaliana, Electrophoresis 30 (2009) 2460–2468.
[35] M. Chaki, R. Valderrama, A. Fernández-Ocaña, A. Carreras, J. López-Jaramillo, F. Luque, J.M. Palma, J.R. Pedrajas, J.C. Begara-Morales, B. Sánchez-Calvo, M.V. Gómez-Rodríguez, F.J. Corpas, J.B. Barroso, Protein targets of tyrosine nitration in sunflower (*Helianthus annuus* L.) hypocotyls, J. Exp. Bot. 60 (2009) 4221–4234.
[36] N. Abello, H.A. Kerstjens, D.S. Postma, R. Bischoff, Protein tyrosine nitration: selectivity, physicochemical and biological consequences, denitration, and proteomics methods for the identification of tyrosine-nitrated proteins, J. Proteome Res. 8 (2009) 3222–3238.
[37] J.M. Souza, G. Peluffo, R. Radi, Protein tyrosine nitration—functional alteration or just a biomarker? Free Radic. Biol. Med. 45 (2008) 357–366.
[38] J.M. Souza, I. Choi, Q. Chen, M. Weisse, E. Daikhin, M. Yudkoff, M. Obin, J. Ara, J. Horwitz, H. Ischiropoulos, Proteolytic degradation of tyrosine nitrated proteins, Arch. Biochem. Biophys. 380 (2000) 360–366.
[39] Y. Kamisaki, K. Wada, K. Bian, B. Balabanli, K. Davis, E. Martin, F. Behbod, Y.C. Lee, F. Murad, An activity in rat tissues that modifies nitrotyrosine-containing proteins, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 11584–11589.
[40] Y. Irie, M. Saeki, Y. Kamisaki, E. Martin, F. Murad, Histone H1.2 is a substrate for denitrase, an activity that reduces nitrotyrosine immuno reactivity in proteins, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 5634–5639.
[41] B. Görg, N. Qvartskhava, P. Voss, T. Grune, D. Häussinger, F. Schliess, Reversible inhibition of mammalian glutamine synthetase by tyrosine nitration, FEBS Lett. 581 (2007) 84–90.
[42] H.S. Smallwood, N.M. Lourette, C.B. Boschek, D.J. Bigelow, R.D. Smith, L. Pasa-Tolić, T.C. Squier, Identification of a denitrase activity against calmodulin in activated macrophages using high-field liquid chromatography—FTICR mass spectrometry, Biochemistry 46 (2007) 10498–10505.

[43] M.C. Romero-Puertas, M. Laxa, A. Mattè, F. Zaninotto, I. Finkemeier, A.M.E. Jones, M. Perazolli, E. Vandelle, K.-J. Dietz, M. Delledonne, S-nitrosylation of peroxiredoxin II E promotes peroxynitrite-mediated tyrosine nitration, Plant Cell 19 (2007) 4120–4130.
[44] D. Galetskiy, J.N. Lohscheider, A.S. Kononikhin, I.A. Popov, E.n. Nikolaev, I. Adamska, Mass spectrometric characterization of photooxidative protein modifications in Arabidopsis thaliana thylakoid membranes, Rapid Commun. Mass Spectrom. 25 (2011) 184–190.
[45] C. Alvarez, J. Lozano-Juste, L.C. Romero, I. García, C. Gotor, J. León, Inhibition of Arabidopsis O-acetylserine(thiol)lyase A1 by tyrosine nitration, J. Biol. Chem. 286 (2011) 578–586.
[46] C. Quijano, D. Hernandez-Saavedra, L. Castro, J.M. McCord, B. A: Freeman, R. Radi, Reaction of peroxynitrite with Mn-superoxide dismutase. Role of the metal center in decomposition kinetics and nitration, J. Biol. Chem. 276 (2001) 11631–11638.
[47] A.M. Cassina, R. Hodara, J.M. Souza, L. Thomson, L. Castro, H. Ischiropoulos, B.A. Freeman, R. Radi, Cytochrome c nitration by peroxynitrite, J. Biol. Chem. 275 (2000) 21409–21415.
[48] Z. Balafanova, R. Bolli, J. Zhang, Y. Zheng, J.M. Pass, A. Bhatnagar, X.-L. Tang, O. Wang, E. Cardwell, P. Ping, Nitric oxide (NO) induces nitration of protein kinase C (PKC), facilitating PKC translocation via enhanced PKC–RACK2 interactions, J. Biol. Chem. 277 (2002) 15021–15027.
[49] A.J. Gow, D. Duran, S. Malcolm, H. Ischiropoulos, Effects of peroxynitrite-induced protein modifications on tyrosine phosphorylation and degradation, FEBS Lett. 385 (1996) 63–66.
[50] S.K. Kong, M.B. Yim, E.R. Stadtman, P.B. Chock, Peroxynitrite disables the tyrosine phosphorylation regulatory mechanism: Lymphocyte-specific tyrosine kinase fails to phosphorylate nitrated cdc2(6-20)NH_2 peptide, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 3377–3382.
[51] H. Ischiropoulos, Biological tyrosine nitration: a pathophysiological function of nitric oxide and reactive oxygen species, Arch. Biochem. Biophys. 356 (1998) 1–11.
[52] G.R. Ross, M. Kang, A.P. Malykhina, N. Shirwany, M. Drozd, H.I. Akbarali, Nitrotyrosylation of calcium channels prevents c-src kinase regulation of colonic smooth muscle contractility in experimental colitis, J. Pharmacol. Exp. Ther. 322 (2007) 948–956.
[53] R. Reinehr, B. Görg, A. Höngen, D. Häussinger, CD95-tyrosine nitration inhibits hyperosmotic and CD95 ligand-induced CD95 activation in rat hepatocytes, J. Biol. Chem. 279 (2004) 10364–10373.
[54] H.P. Monteiro, R.J. Arai, L.R. Travassos, Protein tyrosine phosphorylation and protein tyrosine nitration in redox signaling, Antioxid. Redox Signal. 10 (2008) 843–890.
[55] C. Mallozzi, A.M.M. Di Stasi, M. Minetti, Peroxynitrite modulates tyrosine-dependent signal transduction pathway of human erythrocyte band 3, FASEB J. 11 (1997) 1281–1290.
[56] W.-Q. Shi, H. Cai, D.-D. Xu, X.-Y. Su, P. Lei, Y.-F. Zhao, Y.M. Li, Tyrosine phosphorylation/dephosphorylation regulates peroxynitrite-mediated peptide nitration, Regul. Pept. 144 (2007) 1–5.
[57] L.O. Klotz, S.M. Schieke, H. Sies, N.J. Holbrook, Peroxynitrite activates the phosphoinositide 3-kinase/Akt pathway in human skin primary fibroblasts, Biochem. J. 352 (2000) 219–225.
[58] K. Takakura, J.S. Beckman, L.A. MacMillan-Crow, J.P. Crow, Rapid and irreversible inactivation of protein tyrosine phosphatases PTP1B, CD45, and LAR by peroxynitrite, Arch. Biochem. Biophys. 369 (1999) 197–207.
[59] C. Mallozzi, A.M. Di Stasi, M. Minetti, Nitrotyrosine mimics phosphotyrosine binding to the SH2 domain of the src family tyrosine kinase lyn, FEBS Lett. 503 (2001) 189–195.
[60] M. Minetti, C. Mallozzi, A.M. Di Stasi, Peroxynitrite activates kinases of the src family and upregulates tyrosine phosphorylation signaling, Free Radic. Biol. Med. 33 (2002) 744–754.
[61] E. Pinzar, T. Wang, M.R. Garrido, W. Xu, P. Levy, S.P. Bottari, Angiotensin II induces tyrosine nitration and activation of ERK1/2 in vascular smooth muscle cells, FEBS Lett. 579 (2005) 5100–5104.
[62] H. Narang, F.A. Dhariwala, M. Krishna, Effect of nitric oxide donor and gamma irradiation on modifications of ERK and JNK in murine peritoneal macrophages, J. Cell Commun. Signal. 1 (2007) 219–226.
[63] N. Kinjo, H. Kawanaka, T. Akahoshi, S. Yamaguchi, D. Yoshida, G. Anegawa, K. Konishi, M. Tomikawa, K. Tanoue, A. Tarnawski, M. Hashizume, Y. Maehara, Significance of ERK nitration in portal hypertensive gastropathy and its therapeutic implications, Am. J. Physiol. Gastrointest. Liver Physiol. 295 (2008) G1016–G1024.
[64] R.P. Webster, D. Brockman, L. Myatt, Nitration of p38 MAPK in the placenta: association of nitration with reduced catalytic activity of p38 MAPK in pre-eclampsia, Mol. Hum. Reprod. 12 (2006) 677–685.
[65] P. Zhang, Y.-Z. Wang, E. Kagan, J.C. Bonner, Peroxynitrite targets the epidermal growth factor receptor, Raf-1, and MEK independently to activate MAPK, J. Biol. Chem. 275 (2000) 22479–22486.
[66] A. Pitzschke, A. Schikora, H. Hirt, MAPK cascade signalling networks in plant defence, Curr. Opin. Plant Biol. 12 (2009) 421–426.
[67] J.S. Beckman, T.W. Beckman, J. Chen, P.A. Marshall, B.A. Freeman, Apparent hydroxyl radical production by peroxynitrite: implications for endothelial injury from nitric oxide and superoxide, Proc. Natl. Acad. Sci. U. S. A. 87 (1990) 1620–1624.
[68] H. Botti, M.N. Möller, D. Steinmann, T. Nauser, W.H. Koppenol, A. Denicola, R. Radi, Distance-dependent diffusion-controlled reaction of •NO and O_2•—at chemical equilibrium with $ONOO^-$, J. Phys. Chem. B. 114 (2010) 16584–16593.
[69] A. Denicola, J.M. Souza, R. Radi, Diffusion of peroxynitrite across erythrocyte membranes, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 3566–3571.
[70] C. Quijano, N. Romero, R. Radi, 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 (2005) 728–741.
[71] M. Delledonne, Y. Xia, R.A. Dixon, C. Lamb, Nitric oxide fuctions as a signal in plant disease resistance, Nature 394 (1998) 585–588.
[72] J. Durner, D. Wendehenne, D.F. Klessig, Defense gene induction in tobacco by nitric oxide, cyclic GMP, and cyclic ADP-ribose, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 10328–10333.
[73] T. Keller, H.G. Damude, D. Werner, P. Doerner, R.A. Dixon, C. Lamb, A plant homolog of the neutrophil NADPH oxidase gp91phox subunit gene encodes a plasma membrane protein with Ca^{2+} binding motifs, Plant Cell 10 (1998) 255–266.
[74] S. Saito, A. Yamamoto-Katou, H. Yoshioka, N. Doke, K. Kawakita, Peroxynitrite generation and tyrosine nitration in defense responses in tobacco BY-2 cells, Plant Cell Physiol. 47 (2006) 689–697.
[75] K. Setsukinai, Y. Urano, K. Kakinuma, H.J. Majima, T. Nagano, Development of novel fluorescence probes that can reliably detect reactive oxygen species and distinguish specific species, J. Biol. Chem. 278 (2003) 3170–3175.
[76] B. Heyne, V. Maurel, J.C. Scaiano, Mechanism of action of sensors for reactive oxygen species based on fluorescein-phenol coupling: the case of 2-[6-(4′-hydroxy)phenoxy-3H-xanthen-3-on-9-yl]benzoic acid, Org. Biomol. Chem. 4 (2006) 802–807.
[77] Z.N. Sun, H.L. Wang, F.Q. Liu, Y. Chen, P.K. Tam, D. Yang, BODIPY-based fluorescent probe for peroxynitrite detection and imaging in living cells, Org. Lett. 11 (2009) 1887–1890.
[78] F. Gaupels, E. Spiazzi-Vandelle, D. Yang, M. Delledonne, Detection of peroxynitrite accumulation in Arabidopsis thaliana during the hypersensitive defense response, Nitric Oxide, doi:10.1016/j.niox.2011.01.009.
[79] A. Clarke, R. Desikan, R.D. Hurst, J.T. Hancock, S.J. Neill, NO way back: nitric oxide and programmed cell death in Arabidopsis thaliana suspension cultures, Plant J. 24 (2000) 667–677.
[80] C. Zhang, K.J. Czymmek, A.D. Shapiro, Nitric oxide does not trigger early programmed cell death events but may contribute to cell-to-cell signaling governing progression of the Arabidopsis hypersensitive response, Mol. Plant Microbe Interact. 16 (2003) 962–972.
[81] K.J. Dietz, Peroxiredoxins in plants and cyanobacteria, Antioxid. Redox Signal., doi:10.1089/ars.2010.3657.
[82] A. Sakamoto, S. Tsukamoto, H. Yamamoto, M. Ueda-Hashimoto, M. Takahashi, H. Suzuki, H. Morikawa, Functional complementation in yeast reveals a protective role of chloroplast 2-Cys peroxiredoxin against reactive nitrogen species, Plant J. 33 (2003) 841–851.
[83] O. Lamotte, K. Gould, D. Lecourieux, A. Sequeira-Legrand, A. Lebrun-Garcia, J. Durner, A. Pugin, D. Wendehenne, Analysis of nitric oxide signaling functions in tobacco cells challenged by the elicitor cryptogein, Plant Physiol. 135 (2004) 516–529.
[84] S. de la Fuente van Bentem, H. Hirt, Protein tyrosine phosphorylation in plants: more abundant than expected? Trends Plant Sci. 14 (2009) 71–76.
[85] M.H. Oh, X. Wang, X. Wu, Y. Zhao, S.D. Clouse, S.C. Huber, Autophosphorylation of Tyr-610 in the receptor kinase BAK1 plays a role in brassinosteroid signaling and basal defense gene expression, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 17827–17832.