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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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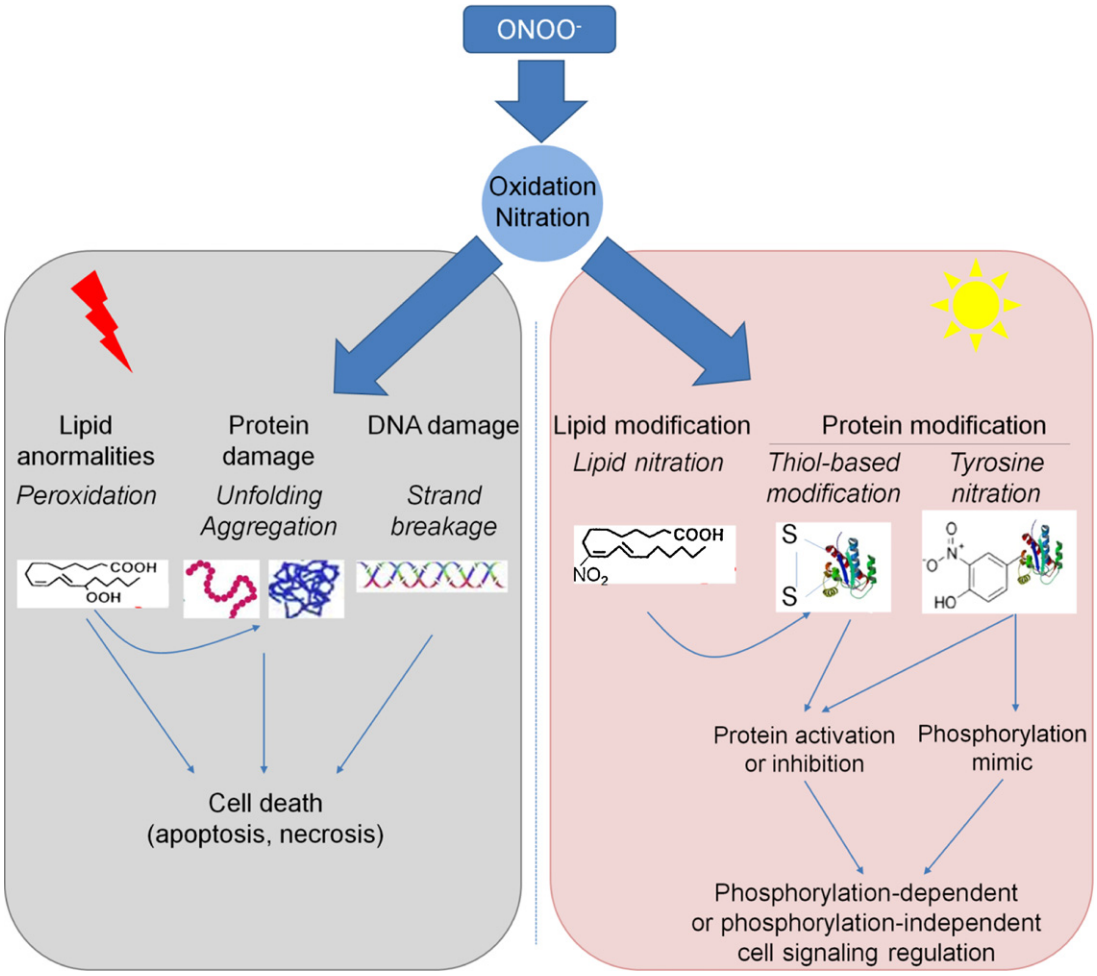

Fig. 1. Dual effects of peroxynitrite on biomolecules. Peroxynitrite triggers a myriad of cytotoxic effects including lipid peroxidation, protein nitration and oxidation, DNA oxidative damage (left panel). If severe enough to overcome cellular antioxidant defenses, the biomolecular injuries initiated 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