Review

Protein tyrosine nitration in plants: Present knowledge, computational prediction and future perspectives

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ABSTRACT

Nitric oxide (NO) and related molecules (reactive nitrogen species) regulate diverse physiological processes mainly through posttranslational modifications such as protein tyrosine nitration (PTN). PTN is a covalent and specific modification of tyrosine (Tyr) residues resulting in altered protein structure and function. In the last decade, great efforts have been made to reveal candidate proteins, target Tyr residues and functional consequences of nitration in plants. This review intends to evaluate the accumulated knowledge about the biochemical mechanism, the structural and functional consequences and the selectivity of plants' protein nitration and also about the decomposition or conversion of nitrated proteins. At the same time, this review emphasizes yet unanswered or uncertain questions such as the reversibility/irreversibility of tyrosine nitration, the involvement of proteasomes in the removal of nitrated proteins or the effect of nitration on Tyr phosphorylation. The different NO producing systems of algae and higher plants raise the possibility of diversely regulated protein nitration. Therefore studying PTN from an evolutionary point of view would enrich our present understanding with novel aspects. Plant proteomic research can be promoted by the application of computational prediction tools such as GPS-YNO$_2$ and iNitro-Tyr software. Using the reference *Arabidopsis* proteome, Authors performed *in silico* analysis of tyrosine nitration in order to characterize plant tyrosine nitroproteome. Nevertheless, based on the common results of the present prediction and previous experiments the most likely nitrated proteins were selected thus recommending candidates for detailed future research.

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1. Biochemical mechanism of protein tyrosine nitration

In general, attach of a nitro group (−NO$_2$) to a chemical compound through a chemical reaction is known as nitration. In

biological systems, fatty acids, nucleic acids and proteins can be targets of such modifications. Despite the fact that in proteins several amino acids such as tyrosine, tryptophan, cysteine and methionine can be affected by nitration, tyrosine nitration got particular attention in both animals and plants partly because besides nitro-tyrosine, the formation of phospho-, chloro-, sulfatyrosine is also feasible (Feeney and Schöneich, 2012).

After the discovery of phosphorylation, in 1992, Ischiropoulos and co-workers first demonstrated the *in vivo* occurrence of protein tyrosine nitration (PTN, Ischiropoulos et al., 1992). Interestingly, as opposed to tyrosine phosphorylation, nitration does not involve enzymatic activity. Regarding the biochemical mechanism, the covalent addition of a nitro group in the *ortho* position of the aromatic ring in tyrosine (Tyr) molecule happens in two steps. The initial step is the formation of tyrosil radical (Tyr•) during the one-electron oxidation of the aromatic ring. The main Tyr oxidants are hydroxyl (OH•) and carbonate ($CO_3^{•-}$) radicals derived from peroxynitrite through at least three pathways (Fig. 1): (1) at suitable pH unstable peroxynitrous acid (ONOOH) is formed by protonation of peroxynitrite, which homolyzes to OH• and •NO_2; (2) at physiological carbon dioxide concentration (1.3 mM) in aqueous environment peroxynitrite reacts with CO_2 generating nitrosoperoxocarboxylate ($ONOOCO_2^-$) which decomposes to carbonate radical and nitrogen dioxide radical (•NO_2); and (3) NO can be oxidized to nitrite (NO_2^-) which together with hydrogen peroxide (H_2O_2) can be metabolized by peroxidases to generate OH• and •NO_2. The oxidation is followed by a radical-radical nitration reaction in which the nitrogen dioxide radical is added to the Tyr• and 3-nitrotyrosine (YNO_2) is being formed (Souza et al., 2008). All the direct *in vivo* oxidants (mainly carbonate and hydroxyl radicals) and nitrating agents (•NO_2) derive from peroxynitrite ($ONOO^-$), which itself is only an indirect contributor to PTN (Yeo et al., 2015; Radi, 2013). Peroxynitrite is formed in the fast reaction between superoxide anion ($O_2^{•-}$) and nitric oxide (NO•); therefore, peroxynitrite derives from NO and consequently it belongs to the group of NO-originated molecules, the reactive nitrogen species (RNS, Patel et al., 1999). Fig. 1 summarizes the chemical reactions leading to the formation of 3-nitrotyrosine.

Superoxide radical anion ($O_2^{•-}$) has a remarkably shorter biological half-life compared to NO (Table 1, Vranova et al., 2002) and because of its negative charge at physiological pH; its diffusion across membranes depends on the presence of anion channels (Denicola et al., 1998). The different diffusion properties of $O_2^{•-}$ and NO suggest that in biological systems, the non-radical anion, peroxynitrite generates close to the sites of $O_2^{•-}$ formation where NO produced at distant cellular spaces arrives (Denicola et al., 1998). Peroxynitrite itself shows longer half-life compared to the other discussed ROS (Siegel et al., 2015, Table 1), but it is more reactive than NO. Regarding the diffusion distance of peroxynitrite, it is similar to that of H_2O_2 and $O_2^{•-}$, but it is shorter compared to NO (Denicola et al., 1998). The direct nitrant, •NO_2 radical has a relatively short half-life and diffusion capability compared to the other reactive nitrogen species (Ford et al., 2002).

2. Fate of nitrated proteins

In order to influence signal transduction independently from phosphorylation routes, tyrosine nitration has to be reversible. This thermodynamically stable modification has earlier been considered to be irreversible but later reductant-dependent and reductant-independent denitrase mechanisms were described in animals (Kuo et al., 1999). Recently, denitrase activity has been characterized in animals (Deeb et al., 2013) and non-enzymatic denitration has also been revealed in case of 8-Nitro-cGMP (Akaike et al., 2010). In plants, denitrase enzyme has not been identified so far, thus the

reversibility of tyrosine nitration remained uncertain. The reduction of the nitro group to amino group resulting 3-aminotyrosine is also conceivable and such reactions may involve nitroreductase activity. Although, bacterial or mammalian nitroreductases proved to be incapable of reducing nitro-tyrosine (Lightfoot et al., 2000). Formation and accumulation of proteasome-resistant protein aggregates can also be conceivable (Hyun et al., 2003). Nitration enhances the susceptibility of the protein for degradation by the proteasome implicating that proteasome functioning is critical for the removal of nitrated proteins (Souza et al., 2000). In plants, it was speculated that nitrated proteins of the roots may be more willing to degrade in 20S proteasomes (Tanou et al., 2012). Castillo et al. (2015) provided recent experimental evidence regarding the role of proteasomes in the degradation of nitrated proteins. In their work, nitrated abscisic acid receptor PYR/PYL/RCAR was polyubiquitylated and consequently it underwent proteasome-regulated degradation.

3. Consequences of tyrosine nitration

Regarding the functional consequences (Fig. 2) of PTN, it leads to the decrease of pK_a of the hydroxyl moiety in the tyrosine residue (from 10–10.3 to 7.2–7.5, Creighton, 1993). Furthermore, nitration of tyrosine enhances the hydrophobicity of the residue and consequently induces structural changes (Souza et al., 2008). A further spatial consequence of PTN originates from the fact that nitrotyrosine is more spacious than tyrosine, which can lead to steric restrictions (Savvides et al., 2002). In plant cells, the available data show that PTN usually causes functional loss of the particular enzyme protein (see Table 2); however the *in vitro* activity of pea glutathione reductase was not affected by this modification (Begara-Morales et al., 2015). In animal systems, PTN-triggered activation, inactivation or no change of activity has been evidenced (Yeo et al., 2015). At the same time, the presence of nitrated tyrosine(s) in a protein is not necessarily the cause of the functional loss, because all biological nitrating agents are also able to exert oxidative effects on amino acids like cysteine or methionine (Alvarez and Radi, 2003).

Another consequence of PTN is the positive or negative impact on tyrosine phosphorylation (Fig. 2), influencing cell signalling as it was observed in non-plant systems (Gow et al., 1996; Kong et al., 1996; Brito et al., 1999; Aburima et al., 2010). In plants, there is no convincing evidence regarding the relationship between tyrosine phosphorylation and nitration. However, recent bioinformatic studies revelaed the presence of tyrosine-specific kinases in the *Arabidopsis* proteome (Carpi et al., 2002), their existence is still controversial (Kovaleva et al., 2013). Both the alteration of tyrosine phosphorylation and nitration causes disturbances in microtubule organization and root hair morphology (Sheremet et al., 2012; Blume et al., 2008) indicating a link between tyrosine phosphorylation and nitration of α-tubulin. It is possible that nitration competes with phosphorylation of α-tubulin for the binding sites (Blume et al., 2008, 2013). Another indirect evidence for the interplay between the two covalent Tyr modifications has been provided by Galetskiy et al. (2011) who revealed that conversely regulated protein phosphorylation and nitration levels control the stability of photosynthetic complexes under high light condition.

4. Specificity and selectivity of tyrosine nitration

Interestingly, only 1–2% of the total tyrosine pool may be the target of *in vivo* nitration (Bartesaghi et al., 2007), suggesting the highly selective nature of the process. This is supported by the low number of YNO_2 sites in plant enzymes containing several tyrosine amino acids (e.g. methionine synthase or monodehydroascorbate