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Review

Biological properties of nitro-fatty acids in plants

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A B S T R A C T

Nitro-fatty acids (NO_2-FAs) are formed from the reaction between nitrogen dioxide (·NO_2) and mono and polyunsaturated fatty acids. Knowledge concerning NO_2-FAs has significantly increased within a few years ago and the beneficial actions of these species uncovered in animal systems have led to consider them as molecules with therapeutic potential. Based on their nature and structure, NO_2-FAs have the ability to release nitric oxide (·NO) in aqueous environments and the capacity to mediate post-translational modifications (PTM) by nitroalkylation. Recently, based on the potential of these ·NO-derived molecules in the animal field, the endogenous occurrence of nitrated-derivatives of linolenic acid (NO_2-Ln) was assessed in plant species. Moreover and through RNA-seq technology, it was shown that NO_2-Ln can induce a large set of heat-shock proteins (HSPs) and different antioxidant systems suggesting this molecule may launch antioxidant and defence responses in plants. Furthermore, the capacity of this nitro-fatty acid to release ·NO has also been demonstrated. In view of this background, here we offer an overview on the biological properties described for NO_2-FAs in plants and the potential of these molecules to be considered new key intermediaries of ·NO metabolism in the plant field.

1. Introduction

A few years ago, interest began to grow regarding the interaction of reactive nitrogen species (RNS), depicted mainly by nitric oxide (·NO) and ·NO-related species such as nitrogen dioxide (·NO_2) or peroxynitrite ($ONOO^-$), with mono- and poly-unsaturated fatty acids. This interaction leads to the formation of nitro-fatty acids, nitrolipids or nitroalkenes (NO_2-FAs) largely characterized by their beneficial role as antioxidant and anti-inflammatory mediators in animal systems [1,15,16,27,34].

Nitroalkenes are present endogenously as free, esterified, and nucleophile-adducted species [11,26,33]. Based on the structure and other properties, NO_2-FAs are ·NO donors [14,18–20,31]. In this respect, the precise mechanism by which these molecules can generate NO has not yet been described. Nevertheless, a putative release has been suggested through a modified Nef reaction with the concomitant formation of a hydroxy-nitroso intermediate being finally able to produce ·NO [31]. Otherwise, a rearrangement in the structure of these ·NO-derived species can also lead to the generation of ·NO [13,18]. Anyhow, this property has conferred to NO_2-FAs the capability of exerting beneficial actions by modifying specific protein targets. In this sense, nitro-linoleic acid (NO_2-LA) mediates the *S*-nitrosylation of CD40, a member of the tumour necrosis factor receptor family, expressed under inflammatory conditions. The *S*-nitrosylation of CD40 leads to its inactivation and is therefore able to trigger an anti-inflammatory response [9].

Moreover, nitro-fatty acids predominantly act via post-translational modification. The presence of a nitroalkene moiety confers electrophilic reactivity to these molecules thus rendering them susceptible to nucleophilic attack by reactive cysteines and histidines [4,29]. This reversible reaction is specifically called nitroalkylation and represents the most widely studied post-translational modification mediated by NO_2-FAs in the animal field. To date, several proteins and transcription factors have been identified as targets of NO_2-FAs resulting in notable antioxidant and anti-inflammatory properties [32]. In this respect, nitro-oleic acid (NO_2-OA) and NO_2-LA mediate nitroalkylation reactions with PPAR-γ, NFκβ or Nrf2/Keap1, among others, thus activating or blunting the activity of these proteins and lately promoting beneficial responses [8,32]. In this context, it is important to bear in mind that under several stressful situations, depicted by inflammatory conditions or disease, such as cardiac ischaemia and reperfusion or ischaemic preconditioning, a significant rise in the levels of NO_2-FAs like NO_2-OA or NO_2-LA has been reported [23,28,30]. The beneficial properties in human health attributed to these molecules may rely on the signalling actions that free NO_2-FAs are able to trigger.

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Although the occurrence and the biological properties of NO_2-FAs have been widely studied in animal systems, very little is known about these molecules in the plant field. In this sense, it has been recently shown that nitro-linolenic acid (NO_2-Ln) is endogenously present in several plant species such as *Arabidopsis thaliana*, *Pisum sativum* or *Oryza sativa* and nitro-conjugated linoleic acid (NO_2-cLA) is in extra-virgin olive oil (EVOO) [10,21]. The application of NO_2-Ln to plants led to determine that NO_2-Ln may trigger a defence mechanism characterized by the induction of a large set of heat-shock proteins (HSPs) and diverse antioxidant systems [21,22]. Based on the growing interest in knowledge of plant NO_2-FAs, here we offer an update of the state of art regarding the biological properties of NO_2-FAs in the plant field and the relevance of understanding their role in plant physiology.

2. Distribution and endogenous occurrence of NO_2-FAs in plants

Recently, the endogenous presence of nitrated derivatives of conjugated linoleic (NO_2-cLA) was firstly identified in extra-virgin olive oil (EVOO), a highly consumed plant-derived product [10]. The strong antioxidant and anti-inflammatory properties attributed to this Mediterranean diet component may be due in part to the presence of NO_2-FAs; thus, based on this discovery, interest in studying the role of these molecules in other plant species has significantly grown. In this regard, as mentioned before, NO_2-Ln was endogenously found in the model plant *Arabidopsis thaliana* [21] and also in other archetypal model plant species with agro-alimentary value such as *Oryza sativa* or *Pisum sativum* [22]. Moreover, the presence of NO_2-FAs was also evaluated in different subcellular fractions of *Pisum sativum* having demonstrated the endogenous occurrence of NO_2-Ln in mitochondria and peroxisome fractions [22]. Results on the quantification of NO_2-Ln in all the species analysed showed the abundance of this molecule varied between 0.07 and 3.8 pmol per gram of fresh weight (pmol/g FW) [22], these data suggesting a potential role as signalling molecules in plant systems.

3. Modulation of the NO_2-FAs content during plant development and under adverse environmental conditions in Arabidopsis

The endogenous occurrence of NO_2-Ln noticed in Arabidopsis prompted a further analysis throughout the developmental process of this model plant. Mass spectrometry approaches showed a modulation on the NO_2-Ln endogenous content with the higher levels being observed at the beginning of plant life (seeds and 14-d-old seedlings) and a continuous decay in the last stages of the life cycle (vegetative and reproductive stages depicted by 30- and 45-d-old, respectively) [21]. Although further studies are needed and because of the capability of NO_2-FAs to act as physiological ·NO donors, it was proposed that the high content of NO_2-Ln at the first stages of plant development may suggest an involvement of this NO_2-FA as a ·NO donor. The highest content of NO_2-Ln noted at the beginning of plant development may suggest a key role of this NO_2-FA at early stages of plant life.

As has previously been reported, different stress conditions lead to a rise in the levels of free NO_2-FAs in animal systems [23,28,30]. With the aim to confirm whether this behaviour took place in plants in the field, several unfavourable abiotic situations such as mechanical wounding, salinity, low temperature, and heavy-metal stress were applied to study the content of NO_2-FAs. Results displayed a significant increase in the levels of NO_2-Ln in all the conditions analysed, both in cell-suspension cultures and in 14-d-old Arabidopsis seedlings [21]. In an effort to understand why this rise in the level of NO_2-FAs occurs under stressful situations, it has been recently demonstrated that an increase of cellular reactive oxygen (ROS) and nitrogen (RNS) species can promote the oxidation of Michael adducts between NO_2-FAs and cysteine residues (Cys) of proteins [24]. Thus, nitro-oxidative stress during abiotic stress conditions can promote the oxidation of nitroalkylated adducts with the subsequent release of free NO_2-FAs, which may lead to the activation of antioxidant and defence responses. Therefore, these results highlight the involvement of NO_2-Ln in plant physiology and in the abiotic stress response, thus suggesting a relevant role of NO_2-FAs also in the plant field.

4. Signalling properties of NO_2-FAs in plant systems

4.1. NO_2-Ln triggers the expression of genes related to stress response

After the endogenous occurrence of NO_2-FAs in plants was ascertained, the potential role of NO_2-Ln in plant physiology was analysed. In this regard, a transcriptomic approach through the RNA-seq technology revealed that NO_2-Ln promotes a significant increase in the expression of a large set of heat-shock proteins (HSPs) and relevant antioxidant components such as the enzymes ascorbate peroxidase (APX) or methionine sulfoxide reductase (MSRB) [21]. This behaviour was previously described in human endothelial cell cultures pre-incubated with NO_2-OA [15], highlighting NO_2-FAs may launch a conserved action mechanism both in the animal and plant field. Recent bioinformatic studies have shown that among the most induced HSR-genes, 52% of the total corresponded to small HSPs, followed by HSP70, HSP40, HSP60, and HSP90 family members. Moreover, an analysis about conserved motifs found on the promoters of NO_2-Ln-over-expressed genes showed the involvement in processes such as the response to stress, cell cycle, and protein synthesis, while conserved motifs present only in the members of the HSR showed that these genes were over-represented in light-induced promoters (Mata-Pérez et al.). These findings highlight the involvement of NO_2-FAs in the response to stress and in diverse cell processes, confirming the relevance of understanding the role of these molecules in plant physiology and stress processes.

4.2. NO_2-Ln is a ·NO donor

Pioneer studies in the field of NO_2-FAs in animal systems showed that NO_2-LA, cholesteryl nitrolinoleate, and nitrohydroxylinoleate can release ·NO [18,31]. This generation takes place preferably in aqueous microenvironments the product being therefore reduced or inhibited in hydrophobic compartments. Later, other studies have confirmed the capability of nitroalkenes acting as ·NO donors but considering this capacity less than 1% of the possible amount of ·NO released *in vitro* [14]. All these results suggest that NO production may be of minor significance *in vivo* compared to the capacity of NO_2-FAs mediating pleiotropic signalling actions by electrophilic-mediated mechanisms [3,12]. Regarding the plant kingdom, the ability of NO_2-Ln, the major NO_2-FA in plants, to release ·NO *in vitro* and *in vivo* has recently been reported [19,20]. Although the precise mechanism of NO release has not been fully understood, it was shown that both NO_2-LA and NO_2-Ln stimulate the generation of ·NO in a similar way. Importantly, a comparison between the capacity of different enzymatic and non-enzymatic ·NO donors led to the finding that NO_2-FAs can promote the generation of ·NO in a similar way to that attributed to the biological ·NO donor, *S*-nitrosoglutathione (GSNO) [19]. These results suggesting that NO_2-Ln in plants can also constitute an important reserve of ·NO in conjunction with S-nitrosothiols (SNOs). Based on the notion that NO_2-FAs are ·NO donors and the relevance of ·NO in living systems, these novel ·NO-derived molecules can be involved in plant development, mediating the response to (a)biotic-stress conditions or through different ·NO-related post-translational modifications (NO-PTMs) through which ·NO transmits its bioactivity [2,5,7,17,25]. In this respect, it has recently shown that APX is a target for NO-PTMs under salinity stress [6]. The *S*-nitrosylation of APX leads to a rise in its activity, suggesting the aforementioned rise in the levels of ROS and RNS taking place under stressful situations can modulate the activity of specific antioxidant systems helping to alleviate oxidative stress. Because NO_2-Ln is a ·NO donor and it is also able to increase the gene and protein expression of APX, it cannot be ruled out that NO_2-Ln can mediate the *S*-nitrosylation of APX,