Environmental and Experimental Botany 93 (2013) 55–62

Contents lists available at SciVerse ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Enhanced lipoxygenase activity is involved in barley root tip swelling induced by cadmium, auxin or hydrogen peroxide

Aster Alemayehu, Beáta Bočová, Veronika Zelinová, Igor Mistrík, Ladislav Tamás*

Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 9, SK-84523 Bratislava, Slovak Republic

A R T I C L E I N F O

Article history:
Received 19 March 2013
Received in revised form 16 May 2013
Accepted 5 June 2013

Keywords:
Auxin
Cadmium
Hydrogen peroxide
Lipoxygenase
Root growth inhibition
Root swelling

A B S T R A C T

Lipoxygenases (EC 1.13.11.12) catalyse the formation of hydroperoxy derivates by oxygenation of polyunsaturated fatty acids. They act as signal molecules, triggering several developmental processes and defence responses under stress conditions. Incubation of Cd-, IAA- or H_2O_2-short-term treated seedlings in the presence of LOX inhibitors efficiently inhibited both Cd-, IAA- or H_2O_2-induced LOX activity and root swelling in a concentration dependent manner, suggesting a key role of LOX or LOX signalling pathway in radial expansion of root cells. Application of antioxidants (ascorbate or N-acetyl cysteine) to the treated seedlings at low 2 mM concentration did not affect the Cd-, IAA- or H_2O_2-induced LOX activity and root swelling. At higher, 10 mM concentration antioxidants markedly inhibited root growth, significantly increased the activity of LOX and evoked the radial expansion of root cells leading to root swelling with well developed root hairs already in control roots. By contrast, the lipophilic antioxidant trolox, a scavenger of hydroperoxides, severely inhibited the development of Cd-, IAA- or H_2O_2-induced root swelling, indicating that not directly LOX, but probably oxylipins, products of LOX pathway, are involved in the induction of root swelling in barley root tip. The results of this study suggest a strong connection between abiotic stress-induced alteration in redox and hormone status caused root growth inhibition and LOX pathway mediated radial expansion of root tip cells.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

It is well known that reactive oxygen and nitrogen species are not only toxic by-products of aerobic metabolism, but undoubtedly play a crucial role in the regulation of different developmental and physiological processes (Gabbita et al., 2000). However, recently an increasing attention has been directed towards to third group of oxidized reactive compounds derived mainly from unsaturated fatty acids. Fatty acid derived reactive electrophilic species represent the largest group of biologically active compounds (oxylipins) ubiquitously occurring in all higher organisms and they are probably involved as signal molecules in all physiological and developmental processes in plants including stress response (Mueller and Berger, 2009). Non-enzymatic lipid peroxides are continuously formed in organism and probably have an evolutionary ancient signal function both in animals and plants (Mueller, 2004). In addition to non-enzymatically formed hydroperoxides, the overwhelming number of oxylipins is produced through the enzymatic pathways, where lipoxygenases (LOX) catalyse the entry reaction forming fatty acid hydroperoxides (Feussner and Wasternack, 2002).

LOXs are ubiquitously distributed enzymes among eukaryotic organisms, which catalyse the formation of hydroperoxy derivates by oxygenation of polyunsaturated fatty acids (Liavonchanka and Feussner, 2006). In plants multiple isoforms of LOXs exist, which have different temporal and spatial distribution during plant development and stress response (Porta and Rocha-Sosa, 2002). Similarly to reactive oxygen and nitrogen species, reactive electrophilic species act as signal molecules, triggering several developmental processes and defence responses, but they are responsible also for cell damage or even for cell death under severe stress conditions (Mueller and Berger, 2009). Furthermore, enhanced LOX activity was also associated with the production of superoxide in senescing bean leaves (Lynch and Thompson, 1984) or with oxidative burst in *Phytophthora sojae*-infected soybean roots (Mithöfer et al., 2002). The increased level of lipid peroxides and LOX activity was also associated with high temperature-induced damages both in leaf and root tissues (Ali et al., 2005). Many studies have also suggested that the elevated LOX activity in the presence of excess heavy metals in plant tissues is a causal factor of enhanced lipid peroxidation and membrane damage (Gallego et al., 1996; Aravind and Prasad, 2003; Zhou et al., 2008; Smeets et al., 2009). On the other hand increasing evidence indicates that the appearance

Abbreviations: IAA, indole-3-acetic acid; ASC, ascorbate; CW, cell wall; LOX, lipoxygenase; NAC, N-acetyl cysteine; PG, propyl gallate; ROS, reactive oxygen species.

* Corresponding author. Tel.: +421 2 59426116; fax: +421 2 54771948.
E-mail address: Ladislav.Tamas@savba.sk (L. Tamás).

0098-8472/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.envexpbot.2013.06.004

of enhanced lipid peroxidation upon exposure of plants to heavy metals does not always result in the oxidative damage of membranes, but may be associated with enhanced LOX activity involved in the initiation of oxylipin pathways. In *Arabidopsis* and *Phaseolus* plants, after Cd or copper treatment a rapid but transient increase of jasmonic acid level occurred suggesting the metal-induced activation of LOX pathway (Maksymiec et al., 2005; Maksymiec, 2011; Cuypers et al., 2011). In addition, enhanced transcript level of LOX has been reported in Cd-exposed seedlings without the enhanced level of lipid peroxidation (Opdenakker et al., 2012).

In our previous work we have demonstrated that the upregulation of LOX is an important component of stress response in barley roots to toxic Cd and it is probably involved in the morphological stress response of root tips or/and in the alleviation of Cd-induced toxic alterations in plant cell membranes, but it is not responsible for the Cd-induced harmful lipid peroxidation and cell death (Liptáková et al., 2013). Therefore, the purpose of this study was to analyse the effect of LOX inhibitors n-propyl gallate (PG) and naproxen, lipid peroxide scavenger trolox and reactive oxygen species (ROS) scavengers ascorbate (ASC) and N-acetyl cysteine (NAC) on LOX activity and Cd, indole-3-acetic acid (IAA) and H_2O_2 stress-induced morphogenic response such as root growth inhibition and radial root swelling in barley root tip.

2. Materials and methods

2.1. Plant material and growth conditions

Barley seeds (*Hordeum vulgare* L.) cv. Slaven (Plant Breeding Station – Hordeum Ltd. **Sládkovičovo**-Nový Dvor) were imbibed in distilled water for 15 min followed by germination between two sheets of filter paper (density $110\,g\,m^{-2}$, Papírna Perštejn, Czech Rep.) moistened with distilled water in Petri dishes. The uniformly germinating seeds, 24 h after the onset of seed imbibition, were arranged into row between two sheets of filter paper moistened with distilled water in rectangle trays. Trays were placed into nearly vertical position to enable downward radical growth. Continuous moisture of filter papers was supplied from the reservoir with distilled water through the filter paper wick. Seedlings, with approximately 4 cm long roots, 60 h after the onset of seed imbibition, were used for treatments.

2.2. Short-term treatments

Roots of barley seedlings were immersed into distilled water (dw – control) or into 15 μM $CdCl_2$, 10 μM IAA (from 1 mM stock in ethanol) or 1 mM H_2O_2 for 30 min. After washing in distilled water for 5 min the seedlings were incubated between two sheets of filter paper moistened with distilled water or with solutions containing 1 or 1.5 mM trolox; 0.25, 0.5 or 1 mM naproxen; 1, 1.5 or 2 mM n-propyl gallate; 2 or 10 mM N-acetyl cysteine and 2 or 10 mM sodium ascorbate as described above. After 6 h of incubation the 4 mm long root tips were used for analysis.

2.3. Root length measurement

For the determination of root length increment the positions of root tips following the treatments were marked on the filter paper. After 6 h, roots were excised at the position of marks and the length increment was measured after recording with stereomicroscope (STMPRO BELPhotonics, Italy) using BEL micro image analyzer. For the localization of root swelling roots were stained with 0.005% Toluidine blue for 5 min and after washing with distilled water were photographed with stereomicroscope.

2.4. Protein extraction and enzyme assays

The root segments were homogenized in a pre-cooled mortar with 0.1 M potassium phosphate extraction buffer (pH 7.8) containing 1 mM EDTA. After centrifugation at $12{,}000 \times g$ for 10 min, proteins were quantified with bovine serum albumin as the calibration standard by the method of Bradford (1976). Lipoxygenase (linoleate:oxygen oxidoreductase; EC 1.13.11.12) activity was measured using the colorimetric method according to Anthon and Barrett (2001). The reaction mixture contained in a final volume of 110 μL 5 mM 3-dimethylaminobenzoic acid in 0.25 M sodium phosphate buffer (pH 6.0), 0.5 mM linoleic acid (from 25 mM stock solution dissolved in Tween 20) and 2.5 μg of proteins from the root extract. The mixture was incubated at 30 °C for 15 min then the mix of 20 μL of 1 mM 3-methyl-2-benzothiazolinone and 20 μL of haemoglobin (500 μg/mL) was added. After 5 min incubation at room temperature the absorbance was measured at 598 nm. To test the possible effects of peroxidases on reaction 1 mM azide (from 10 mM water stock solution) was added into the reaction mixture to inhibit non-specific peroxidase activity.

2.5. Statistical analyses

The experiments were carried out in five independent series with three replicates (30 root tips per replicate). The data were analysed by one-way analysis of variance (ANOVA test), and the means were separated using Tukey's test.

3. Results and discussion

3.1. Effect of LOX inhibitors on Cd-induced morphogenic responses and LOX activity

Inhibition of root growth is the most widely observed symptom of various abiotic stresses, including heavy metals. In addition to root growth inhibition evoked by the short-term treatment of roots with 15 μM Cd (Fig. 1A), radial expansion of cortical cells and accelerated root hair development in the elongation zone (during treatment) of root tips was detected appearing as a visible root swelling with long root hairs 6 h after the short-term treatment (Fig. 1C). Short-term Cd treatment of barley roots also led to the induction of LOX, representing a twofold increase in the activity at 6 h after treatment in comparison with the control root tips (Fig. 1B). The peroxidase inhibitor KCN had no significant effect on enzymatic reaction (controls: without KCN 0.512 ± 0.048 or with KCN 0.521 ± 0.061 and Cd – without KCN 0.932 ± 0.032 or with KCN 0.911 ± 0.069; similar results were observed in the case of IAA or H_2O_2 – data not shown) suggesting that it was catalysed by LOX but not by non-specific peroxidases. In addition, our previous studies have shown that two of three LOX isozymes are strongly stimulated by Cd and were insensitive to KCN (Liptáková et al., 2013). Several observations suggest that LOXs are involved in various developmental processes and in responses to stress conditions.

Incubation of Cd-short-term treated seedlings at the presence of LOX inhibitor naproxen efficiently inhibited both Cd-induced LOX activity and root swelling in a concentration dependent manner (Fig. 1), suggesting a key role of LOX or LOX signalling pathway in radial expansion of root cells. In contrast to root swelling, in the case of root growth a synergistic effect of Cd and naproxen action was observed, resulting in a more severe root growth inhibition than in Cd or naproxen treatment alone (Fig. 1A). In control seedlings, exposed to distilled water for 30 min and subsequently incubated in the presence of naproxen, a significant decrease of LOX activity and a marked root growth inhibition was observed especially at the higher concentrations of naproxen. In spite of a slight decrease in

Download English Version:

https://daneshyari.com/en/article/6388953

Download Persian Version:

https://daneshyari.com/article/6388953

Daneshyari.com