Journal of Plant Physiology 209 (2017) 68–75

Contents lists available at ScienceDirect

Journal of Plant Physiology

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

Mitochondrial complex II-derived superoxide is the primary source of mercury toxicity in barley root tip

Ladislav Tamás*, Veronika Zelinová

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

ARTICLE INFO

Article history:
Received 9 August 2016
Received in revised form 18 October 2016
Accepted 19 October 2016
Available online 5 December 2016

Keywords:
Barley root
Cell death
Mercury
Reactive oxygen species
Succinate dehydrogenase
Superoxide

ABSTRACT

Enhanced superoxide generation and significant inhibition of succinate dehydrogenase (SDH) activity followed by a strong reduction of root growth were detected in barley seedlings exposed to a 5 μM Hg concentration for 30 min, which increased further in an Hg dose-dependent manner. While at a 25 μM Hg concentration no cell death was detectable, a 50 μM Hg treatment triggered cell death in the root meristematic zone, which was markedly intensified after the treatment of roots with 100 μM Hg and was detectable in the whole root tips. Generation of superoxide and H_2O_2 was a very rapid response of root tips occurring even after 5 min of exposure to Hg. Application of an NADPH oxidase inhibitor or the inhibition of electron flow in mitochondria by the inhibition of complex I did not influence the Hg-induced H_2O_2 production. Treatment of roots with thenoyltrifluoroacetone, a non-competitive inhibitor of SDH, markedly reduced root growth and induced both superoxide and H_2O_2 production in a dose dependent manner. Similar to results obtained in intact roots, Hg strongly inhibited SDH activity in the crude mitochondrial fraction and caused a considerable increase of superoxide production, which was markedly reduced by the competitive inhibitors of SDH. These results indicate that the mitochondrial complex II-derived superoxide is the primary source of Hg toxicity in the barley root tip.

© 2016 Elsevier GmbH. All rights reserved.

1. Introduction

We must currently face the reality that the pollution of the environment as a negative effect of industrialization is a global problem. Heavy metals are the extremely dangerous substances between the pollutants due to their stability and toxicity even at very low concentrations. Mercury (Hg) is one of the most toxic environmental contaminants due to its low melting and boiling points, conversion between chemical forms and participation in several biological cycles. Moreover, the global atmospheric Hg deposition rate is currently approximately three times higher than during pre-industrial times (Hylander and Meili, 2003). However, a major sink for heavy metals, including Hg, is the pedosphere. Therefore, their concentrations in the soils may exceed several times the non-toxic level to most plant species (Han et al., 2002). In addition, many fertilizers, pesticides and various soil amendments contain a considerable amount of Hg, and thus directly contaminate arable soils.

Hg is easily taken up by plant cells through the various transport systems for essential micronutrients (Esteban et al., 2008) and is mainly accumulated in the roots, interfering with several physiological and developmental processes. At the same time, a growing body of evidence indicates that the Hg-induced oxidative stress in cells plays a pivotal role in the development of Hg toxicity symptoms, including root growth inhibition (Chen and Yang, 2012). Numerous publications have described the elevated levels of lipid peroxides and oxidized proteins in both roots and shoots of various crop plants, leading to reduced biomass production (Cho and Park, 2000; Cargnelutti et al., 2006; Rellán-Álvarez et al., 2006; Zhou et al., 2007). The rapid activation of protective antioxidative defense mechanisms is a prerequisite for the successful detoxification of reactive oxygen species (ROS) in a toxic overdose. Therefore, the increased level of both enzymatic and non-enzymatic antioxidant systems are a common root response to elevated Hg level (Zhou et al., 2008; Sobrino-Plata et al., 2009; Lomonte et al., 2010). Because moderate ROS levels act as signals in the regulation of stress responses, Hg upregulated several genes encoding proteins involved in the defense responses of plants to both biotic and abiotic stresses (Didierjean et al., 1996; Sävenstrand and Strid, 2004).

Due to the very high affinity of Hg to the sulfhydryl groups, the phytochelatins play a key role in the detoxification of Hg in the cytosol, forming phytochelatin-Hg multicomplexes (Iglesia-

Abbreviations: DPI, diphenyleneiodonium; mROS, mitochondrial ROS; ROS, reactive oxygen species; SDH, succinate dehydrogenase; TIFA, thenoyltrifluoroacetone.

* Corresponding author.
E-mail address: Ladislav.Tamas@savba.sk (L. Tamás).

http://dx.doi.org/10.1016/j.jplph.2016.10.014
0176-1617/© 2016 Elsevier GmbH. All rights reserved.

Turiño et al., 2006). Analysis of the soluble fraction of barley, maize and alfalfa roots exposed to Hg revealed that Hg was only associated with the phytochelatins (Carrasco-Gil et al., 2011). A transgenic *Arabidopsis* line with increased phytochelatin content showed enhanced tolerance to Hg in comparison to the wild type seedlings (Li et al., 2006). On the other hand, just due to this high affinity of Hg to the sulfhydryl groups, the various biomolecules with these moieties are the main targets of Hg toxicity (Patra et al., 2004). In photosynthetic cells, the chloroplast and peroxisome are the main sources of ROS production, while in roots or in the dark the mitochondria are the main sources of ROS production (Rhoads et al., 2006). Both chloroplastic and mitochondrial electron transport chains contain numerous proteins with high amounts of sulfhydryl groups, which, just after their modification or inhibition may generate a large amount of ROS. It has been well documented that Hg inhibits photosynthetic electron transport at multiple sites in a concentration dependent manner (Murthy and Mohanty, 1993; Bernier and Carpentier, 1995). Recently, numerous publications have reported the mitochondrial origin of increased ROS production in several plant species exposed to toxic concentrations of different metals (Keunen et al., 2011). However, the precise mechanism of Hg-induced ROS generation in roots remains unclear. In human monocytic cells, Hg markedly inhibited mitochondrial activity via oxidative stress (Messer et al., 2005). Furthermore, succinate dehydrogenase (SDH) as a possible target site of Hg toxicity has been suggested in fish liver and rat brain (Rao et al., 2010; Mieiro et al., 2015). SDH has been described as a source of ROS and as a regulator of development and stress, including defense responses in plants (Huang and Millar, 2013; Jardim-Messeder et al., 2015). An Arabidopsis mutant in the complex II subunit showed considerably reduced SDH activity and ROS production and exhibited increased susceptibility to pathogens, suggesting that ROS generated by mitochondrial SDH are involved in the activation of defense response to biotic stresses (Gleason et al., 2011).

The aim of the present study was to analyze the possible involvement of mitochondrial SDH in ROS generation in the responses of the barley root tip to Hg stress.

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, Slovakia) were imbibed in distilled water for 15 min followed by germination between two sheets of filter paper (density 110 g/m^2) moistened with distilled water in Petri dishes at 25 °C in darkness. The uniformly germinating seeds, 24 h after the onset of seed imbibition, were arranged into rows between two sheets of filter paper moistened with distilled water in rectangle trays. Trays were placed into a 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 primary roots, 60 h after the onset of seed imbibition, were used for treatments.

2.2. Short-term treatments

During the short-term treatments, roots were immersed into appropriate test solutions, such as distilled water (dw; control; pH 5.5), 1–100 μM $HgCl_2$, pH 5.2–5.5 depending on Hg concentration, 100–500 μM TIFA, pH 5–5.5 depending on TIFA concentration (500 mM stock in DMSO, the final concentration of DMSO was 0.1%), 1 μM diphenyleneiodonium – DPI, pH 5.4 (10 mM stock in DMSO, the final concentration of DMSO was 0.1%), or 5 μM rotenone, pH 5.5 (4 mM stock in methanol, the final concentration of methanol was 0.25%), for 5–30 min. Following the rinse in dw for 5 min, the seedlings (3 mm long root tips) were immediately used for analysis or were incubated between two sheets of filter paper moistened with distilled water as described above for 6 or 9 h and used for root length measurement and cell death analysis.

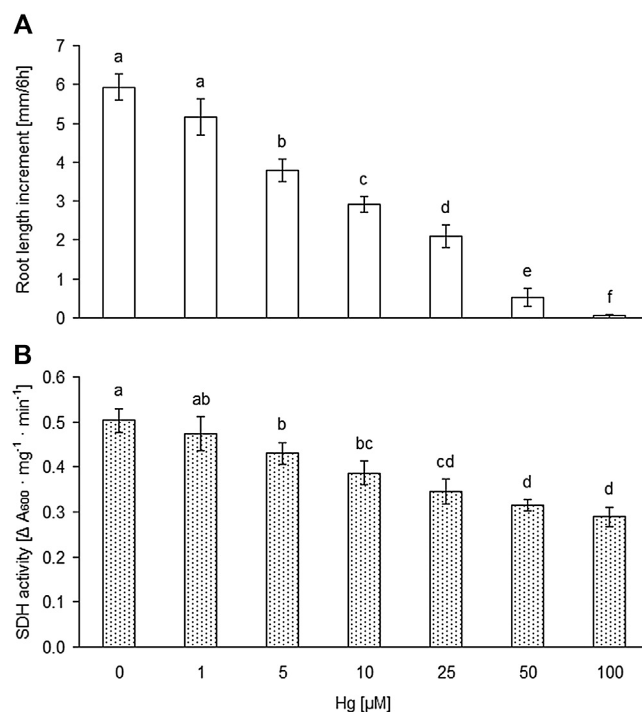

Fig. 1. Root length increments 6 h (A) and SDH activity (B) immediately after the short-term treatment of roots with 0–100 μM Hg for 30 min. Mean values ± SD (n = 5). Different letters indicate statistical significance according to Tukey's test (P < 0.05).

2.3. Root length measurement

For the determination of root length changes, the position of root tips following the treatments was marked on the filter paper. After 6 h, the roots were excised at the position of marks, and the change in length was measured after recording with a stereomicroscope using an image analyzer. For localization of root swelling, the roots were stained with 0.05% Toluidine blue for 10 min and after washing with distilled water were photographed with stereomicroscope.

2.4. Measurement of hydrogen peroxide production by root tips

H_2O_2 production was monitored fluorimetrically using the Amplex Ultra Red Hydrogen Peroxide Assay Kit (Molecular Probes) according to manufacturer's recommendations, with minor modifications. Segments (3 mm) from barley root tips (20 segments per reaction) were washed in 400 μl of 20 mM sodium phosphate buffer, pH 6.0, for 5 min. After this washing, the root tips were incubated in 400 μl of 20 mM sodium phosphate buffer, pH 6.0, containing 50 μM Amplex UltraRed reagent (from 10 mM DMSO stock solution) and 0.1 U of horseradish peroxidase for 15 min at 30 °C. The fluorescence signal was recorded (300 μl of reaction mixture without root segments) with the microplate reader using excitation at 485 (filter 485/20) nm and fluorescence detection at 590 (filter 590/20) nm. H_2O_2 production was expressed as an increase in relative fluorescence unit (RFU) during 15 min incubation of root

Download English Version:

https://daneshyari.com/en/article/5518193

Download Persian Version:

https://daneshyari.com/article/5518193

Daneshyari.com