Journal of Plant Physiology 191 (2016) 63–72

Contents lists available at ScienceDirect

Journal of Plant Physiology

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

Physiology

The auxin conjugate indole-3-acetyl-aspartate affects responses to cadmium and salt stress in *Pisum sativum* L.

Maciej Ostrowski*, Anna Ciarkowska, Anna Jakubowska

Nicolaus Copernicus University, Department of Biochemistry, Lwowska 1 87-100 Torun, Poland

ARTICLE INFO

Article history:
Received 25 August 2015
Received in revised form
25 November 2015
Accepted 25 November 2015
Available online 14 December 2015

Keywords:
Auxin conjugate
Indole-3-acetyl-aspartate
Salt stress
Heavy metal stress
Ascorbate peroxidase
Guaiacol peroxidase
Catalase
Protein carbonylation
P. sativum

ABSTRACT

The synthesis of IAA-amino acid conjugates is one of the crucial regulatory mechanisms for the control of auxin activity during physiological and pathophysiological responses. Indole-3-acetyl-aspartate (IAA-Asp) is a low molecular weight amide conjugate that predominates in pea (*Pisum sativum* L.) tissues. IAA-Asp acts as an intermediate during the auxin degradation pathway. However, some recent investigations suggest a direct signaling function of this conjugate in various processes. In this study, we examine the effect of 100 μM IAA-Asp alone and in combination with salt stress (160 mM NaCl) or heavy metal stress (250 μM $CdCl_2$) on H_2O_2 concentration, protein carbonylation as well as catalase and ascorbate (APX) and guaiacol peroxidase (GPX) activities in 7-day-old pea seedlings. As revealed by spectrophotometric analyses, IAA-Asp increased the carbonylated protein level and reduced the H_2O_2 concentration. Moreover, IAA-aspartate potentiated the effect of both Cd^{2+} ions and NaCl on the H_2O_2 level. The enzymatic activities (catalase and peroxidases) were examined using spectrophotometric and native-PAGE assays. IAA-Asp alone did not affect catalase activity, whereas the two peroxidases were regulated differently. IAA-Asp reduced the APX activity during 48 h cultivation. APX activity was potentiated by IAA-Asp + NaCl after 48 h. Guaiacol peroxidase activity was diminished by all tested compounds. Based on these results, we suggest that IAA-Asp can directly and specifically affect the pea responses to abiotic stress.

© 2015 Elsevier GmbH. All rights reserved.

1. Introduction

Most of the IAA in plants is found not as the free (active) form, but conjugated to sugars, *myo*-inositol and high molecular mass polysaccharides via an ester linkage or to amino acids, peptides and proteins by amide linkage (Bandurski et al., 1995; Ludwig-Müller, 2011). IAA conjugation is one of possible mechanisms for regulation of the free auxin levels depending on a variety of developmental and environmental factors (reviewed in Woodward and Bartel, 2005).

It is widely assumed that dicotyledonous plants, including pea (*Pisum sativum*), accumulate IAA-amide conjugates in which IAA is bound to single amino acids, peptides or small proteins (Slovin et al., 1999; Walz et al., 2002; Park et al., 2006). Indole-3-acetyl-L-aspartate (IAA- aspartate, IAA-Asp) is the most common amide linkage of auxin in pea tissues (Andrea and Good, 1955; Südi, 1964). In pea seeds, the level of IAA-amide conjugates with IAA-Asp as the predominant form of auxin is 2.2-fold higher than the IAA level, and IAA-ester levels are below the detection limit (Bandurski and Schulze, 1977).

Substantial evidence indicates that certain IAA-amide conjugates, such as IAA-Ala, IAA-Leu, IAA-Phe, IAA-Val can contribute to the pool of free IAA through the action of IAA-amido hydrolases (Hangarten and Good, 1981; Bartel and Fink, 1995; Ludwig-Müller et al., 1996; Davies et al., 1999; LeClere et al., 2002) In contrast, IAA-Asp and probably IAA-Glu do not serve as a reservoir of IAA but can have more complex functions in plants IAA-Asp is a very poor substrate for a small family of *Arabidopsis thaliana* hydrolases (LeClere et al., 2002) and no specific plant enzyme releasing IAA from IAA-Asp has yet been found. Only Ludwig-Müller et al. (1996) described the hydrolysis of IAA-Asp in the root of Chinese cabbage during *Plasmodiaphora brassicae* infection. The catalytic activity of several *Medicago truncatula* amidohydrolases releasing IAA from IAA-Asp also increased during interaction with symbionts, as reported by Campanella et al. (2008). It is well documented that IAA-Asp is

Abbreviations: ABA, abscisic acid; AEC, 3-amine-9-ethylcarbazole; APX, ascorbate peroxidase; CAT, catalase; DNP, dinitrophenol; FIN219, far-red insensitive 219; GH3, Gretchen Hagen 3; GPX, guaiacol peroxidase; IAA, indole-3-acetic acid; IAA-Asp, indole-3-acetyl-aspartate; ICA-Cys, indole-3-carboxyl-cysteinate; JA, jasmonic acid; JAR1, jasmonate resistance 1; PAGE, polacrylamide gel electrophoresis; PCD, programmed cell death; POX, peroxidase; ROS, reactive oxygen species; SA, salicylic acid; SA-Asp, salicyloyl-aspartate; TIR 1, transport inhibitor response 1.

* Corresponding author. Fax: +48 56 611 47 76.
E-mail address: maciejost@umk.pl (M. Ostrowski).

http://dx.doi.org/10.1016/j.jplph.2015.11.012
0176-1617/© 2015 Elsevier GmbH. All rights reserved.

linked to the IAA inactivation mechanism by oxidation of the bound IAA (Östin et al., 1992,1998; Kai et al., 2007). The results reported by Oetiker and Aeschbacher (1997) suggest that the conjugation of IAA to aspartate has unique physiological significance. A high temperature resistant cell line of henbane (*Hyoscyamus muticus*) accumulated IAA-Asp, whereas IAA-Glc was the main conjugate in a temperature-sensitive cell line. Moreover, some results support the possibility that IAA- Asp conjugate is involved in the regulation of IAA levels in different developmental processes. For example, the reduction of the IAA level by conjugation to IAA-Asp appears to be an important element of auxin homeostasis during the maturation of mung bean seeds (Liu et al., 2007), the ripening climacteric and non-climacteric fruits (Liu et al., 2005; Böttcher et al., 2010, 2013; Böttcher et al., 2013) as well as during embryo development in multi-seed fruits of *Viciae* species (Slater et al., 2013). Recently, González-Lamothe et al. (2012) suggested a direct biological function of IAA-Asp in the promotion of disease development in *A. thaliana* probably by inducing pathogen virulence or growth. Finally, IAA-Asp itself may represent a biologically active molecule with a role that has not yet been identified In contrast to the active amide conjugate of jasmonic acid (JA), jasmonoyl-L-isoleucine (JA-Ile) (Katsir et al., 2008), there is no evidence that IAA-Asp or any other IAA conjugate acting as a signaling molecule is recognized by a specific receptor. However, the possibility that some amide conjugates, such as JA-Trp and IAA-Trp as well as some α-alkyl modified IAAs could act as anti-auxin agents by blocking of IAA binding to the TIR1/AFB receptor has been reported (Hayashi et al., 2008; Staswick, 2009).

The amide conjugates of auxin are synthesized by acyl- adenylate forming/amidosynthetase enzymes, which are encoded by one class of early-auxin responsive genes that belong to the *GH3* (*Gretchen Hagen 3*) family (Hagen et al., 1984; Staswick et al., 2005). These enzymes exist as cytoplasmic, monomeric 67–72 kDa proteins, which catalyze ATP-dependent conversion of IAA to IAA-amino acid (Staswick et al., 2005; Ludwig-Müller et al., 2009; Westfall et al., 2010; Ostrowski et al., 2014; Wang et al., 2015). Since the isolation of the first auxin-responsive *GH3* gene from soybean (*Glycine max*) (Hagen et al., 1984), members of the *GH3* family have been identified in many plant species including tobacco (*Nicotiana tabaccum)* (Roux and Perrot-Rechenmann, 1997), apple (*Malus domestica)* (Devoghalaere et al., 2012), *A. thaliana* (Staswick et al., 2002), calamondin (*Citrus madurensis*) (Lahey et al., 2004), moss (*Physcomitrella patens*) (Bierfreund et al., 2004), alfalfa (*M. truncatula*) (Yang et al., 2015), pungent pepper (*Capsicum chinense*) (Liu et al., 2005), rice (*Oryza sativa)* (Jain et al., 2006), pine (*Pinus pinaster*) (Reddy et al., 2006), tomato (*Solanum lycopersicum* L.) (Kumar et al., 2012) and grapevine (*Vitis vinifera* L.) (Böttcher et al., 2010, 2013).

It is well documented that the expression of many *GH3* genes is affected by various abiotic and biotic factors. For example, *OsGH3-2* transcription was up- and down-regulated by drought and cold, respectively (Du et al., 2012). *OsGH3.13/TLD1* encoding IAA-Asp conjugating enzyme was found to be a positive regulator of drought stress tolerance (Zhang et al., 2009) and overexpression of another *OsGH3* homologue, *OsGH3-1* resulted in an enhanced resistance to a fungal pathogen infection (Domingo et al., 2009). Several tomato *GH3* genes were induced by cold and salt stress conditions (Kumar et al., 2012). Additionally, some tomato *GH3* genes were shown to be elements of the mycorrhizal signaling pathway (Liao et al., 2014). Park et al. (2007) hypothesized that *AtGH3.5/WES-1* can modulate auxin homeostasis in stress adaptation. Recently, Salopek-Sondi et al. (2013) reported down-regulation of *Brassica rapa GH3.1* and *GH3.9* transcripts by stress hormones: ABA, JA, and SA. Similarly, the effects of ABA, JA, SA as well as auxinic herbicide and light on *GH3* expression in pea seedlings have been shown (Ostrowski and Jakubowska, 2013). The expression patterns of *GH3* family genes in maize are also responsive to several abiotic stresses phytohormones as reported by Feng et al. (2014).

It is well known that stress conditions, such as salinity, drought, heavy metals and pathogen infections elicit oxidative stress (Moran et al., 1994; Krishnamurthy and Rathinasabapathi, 2013; Yuan et al., 2013) inducing an increase of levels of reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), superoxide anion (O_2^-), hydroxyl radical ($^\bullet OH$) and hydrogen peroxide (H_2O_2). H_2O_2 is degraded through antioxidant enzymes, such as peroxidase (POX) and catalase (CAT). Additionally, exogenous application of auxin affects the level of H_2O_2 as well as CAT and POX enzymatic activities in plant tissues (Joo et al., 2001; Tyburski et al., 2009; Peer et al., 2013). It seems to be a specific response, because the double auxin receptor mutant *tir1/afb2* exhibited diminished H_2O_2 concentration and enhanced catalase and ascorbate peroxidase activities (Iglesias et al., 2010; Krishnamurthy and Rathinasabapathi, 2013).

In this study we address the potential role of IAA-Asp, non-hydrolyzable auxin conjugate, in responses to environmental abiotic stresses (salt and heavy metal). IAA-Asp affects the activity of some enzymes involved in oxidative stress (ascorbate peroxidase, guaiacol peroxidase, catalase), H_2O_2 concentration and may alter protein carbonylation levels during cultivation of pea seedlings on NaCl and $CdCl_2$. We suggest that IAA-Asp can modulate the effects of NaCl and $CdCl_2$ on abiotic stress effectors in pea seedlings.

2. Material and methods

2.1. Plant material and determination of plant growth

Seeds of pea (*P. sativum* L.) were soaked in distilled water at room temperature for 4 h. Plants were grown on water in darkness at 22–25 °C for 7 days at Petri dishes. Next, the etiolated pea seedlings were transferred to Petri dishes and cultured on water (control) or on the solutions containing 100 μM IAA-Asp (Olchemim, Czech Republic), 160 mM NaCl, 250 μM $CdCl_2$, 100 μM IAA-Asp with 160 mM NaCl, and 100 μM IAA-Asp with 250 μM $CdCl_2$ (Fusconi et al., 2006). After transfer, the cultures were continued for 24–48 h in darkness (control, 160 mM NaCl, 100 μM IAA-Asp, and 100 μM IAA-Asp with 160 mM NaCl, 250 μM $CdCl_2$, 100 μM IAA-Asp with 250 μM $CdCl_2$). Root and stem lengths were measured for 9 plants per each treatment.

2.2. Quantitative measurement of protein carbonyl groups

The concentration of protein carbonyl groups was determined spectrophotometrically according to the method of Dębska et al. (2013). 0.5 g of pea seedlings were homogenized in 5 mL of 0.1 M Tris–HCl buffer pH 7.0 containing 1 mM EDTA, 2% (w/v) PVPP, 1% (w/v) Protease Inhibitor Cocktail (Sigma–Aldrich) and 5 mM DTT using UltraTurax T25 homogenizer (IKA, Germany). The homogenates were centrifuged at 15,000 × *g* for 15 min, (4 °C, 12156 rotor, Sigma Sartorius 3 K 30Centrifuge). Next, the supernatant was filtered through the cotton wool and streptomycine sulphate to a final concentration of 1% (w/v) was added. Then, the mixture was incubated in the dark for 20 min at room temperature. Aliquots of the supernatant containing 0.5 mg protein were incubated with 500 μL 10 mM 2,4-dinitrophenylhydrazine (DNPH) (Sigma–Aldrich) in 2 M HCl in the dark for 35 min at 37 °C. Control samples (without DNPH) were incubated in 500 μL of 2 M HCl. Proteins were precipitated for 10 min with 500 μL of 20% (w/v) TCA and the pellets were washed 3 times with 1:1 (v/v) ethanol:ethyl acetate. After each washing step, the samples were centrifuged for 5 min at 10,000 × *g*. Washed pellets were dissolved in 6 M guanidine hydrochloride in 2 M HCl. The absorbance was measured at

Download English Version:

https://daneshyari.com/en/article/2055467

Download Persian Version:

https://daneshyari.com/article/2055467

Daneshyari.com