Journal of Plant Physiology 171 (2014) 53–62

Contents lists available at ScienceDirect

Journal of Plant Physiology

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

Physiology

Hydrogen sulfide delays GA-triggered programmed cell death in wheat aleurone layers by the modulation of glutathione homeostasis and heme oxygenase-1 expression

Yanjie Xie[1], Chen Zhang[1], Diwen Lai, Ya Sun, Muhammad Kaleem Samma, Jing Zhang, Wenbiao Shen*

College of Life Sciences, Co. Laboratory of Nanjing Agricultural University and Carl Zeiss Far East, Nanjing Agricultural University, Nanjing 210095, Jiangsu Province, China

ARTICLE INFO

Article history:
Received 18 April 2013
Received in revised form 1 September 2013
Accepted 2 September 2013
Available online 16 November 2013

Keywords:
Glutathione
Heme oxygenase-1
Hydrogen sulfide
Programmed cell death
Wheat aleurone layers

ABSTRACT

Hydrogen sulfide (H_2S) is considered as a cellular signaling intermediate in higher plants, but corresponding molecular mechanisms and signal transduction pathways in plant biology are still limited. In the present study, a combination of pharmacological and biochemical approaches was used to study the effect of H_2S on the alleviation of GA-induced programmed cell death (PCD) in wheat aleurone cells. The results showed that in contrast with the responses of ABA, GA brought about a gradual decrease of L-cysteine desulfhydrase (LCD) activity and H_2S production, and thereafter PCD occurred. Exogenous H_2S donor sodium hydrosulfide (NaHS) not only effectively blocked the decrease of endogenous H_2S release, but also alleviated GA-triggered PCD in wheat aleurone cells. These responses were sensitive to hypotaurine (HT), a H_2S scavenger, suggesting that this effect of NaHS was in an H_2S-dependent fashion. Further experiment confirmed that H_2S, rather than other sodium- or sulphur-containing compounds derived from the decomposing of NaHS, was attributed to the rescuing response. Importantly, the reversing effect was associated with glutathione (GSH) because the NaHS triggered increases of endogenous GSH content and the ratio of GSH/oxidized GSH (GSSG) in GA-treated layers, and the NaHS-mediated alleviation of PCD was markedly eliminated by L-buthionine-sulfoximine (BSO, a selective inhibitor of GSH biosynthesis). The inducible effect of NaHS was also ascribed to the modulation of heme oxygenase-1 (HO-1), because the specific inhibitor of HO-1 zinc protoporphyrin IX (ZnPP) significantly suppressed the NaHS-related responses. By contrast, the above inhibitory effects were reversed partially when carbon monoxide (CO) aqueous solution or bilirubin (BR), two of the by-products of HO-1, was added, respectively. NaHS-triggered HO-1 gene expression in GA-treated layers was also confirmed. Together, the above results clearly suggested that the H_2S-delayed PCD in GA-treated wheat aleurone cells was associated with the modulation of GSH homeostasis and HO-1 gene expression.

Introduction

Ample evidence illustrates that programmed cell death (PCD) is an actively controlled, genetically encoded self-destructive mechanism of the cell, which occurs at all stages of life cycle, from fertilization of the ovule to cell death (Jacobson, 1997; Danial and Korsmeyer, 2004). In fact, PCD is not only a central theme during the normal developmental program of many plant species, but also a crucial component of a plant's responses to biotic and abiotic stress (Jabs, 1999). Among these, the cereal aleurone layers really provide a convenient model system for studying plant PCD following germination, which is stimulated by gibberellic acid (GA), while prevented by abscisic acid (ABA) (Kuo et al., 1996; Bethke et al., 1999; Fath et al., 2000, 2002; Domínguez et al., 2004).

Molecular control of plant PCD process has been elucidated previously. It is well known that reactive oxygen species (ROS)

Abbreviations: ABA, abscisic acid; APX, ascorbate peroxidase; BR, bilirubin; BSO, L-buthionine-sulfoximine; BV, biliverdin IX; CAT, catalase; CO, carbon monoxide; CSE, cystathionine γ-lyase; FDA, fluorescein diacetate; FM 4-64, N-(3-triethylammoniumpropyl)-4-(6-[4-(diethylamino) phenyl] hexatrienyl) pyridinium dibromide; GA, gibberellic acid; GSH, glutathione; GSSG, oxidized glutathione; HCOOH, formic acid; HO, heme oxygenase; HO-1, heme oxygenase-1; HSP, heat shock protein; HT, hypotaurine; H_2O_2, hydrogen peroxide; H_2S, hydrogen sulfide; LCD, L-cysteine desulfhydrase; NaHS, sodium hydrosulfide; NO, nitric oxide; PCD, programmed cell death; ROS, reactive oxygen species; ZnPP, zinc protoporphyrin IX.

* Corresponding author. Tel.: +86 258 4399032; fax: +86 258 4396542.
E-mail addresses: wbshenh@njau.edu.cn, 13851857655@139.com (W. Shen).

[1] These authors contributed equally to this work.

0176-1617/$ – see front matter © 2013 Elsevier GmbH. All rights reserved.
http://dx.doi.org/10.1016/j.jplph.2013.09.018

modulate expression of a number of genes and therefore control PCD processes (Jabs, 1999; Fath et al., 2001; Torres and Dangl, 2005). In plants, ROS homeostasis was maintained by an efficient non-enzymatic (glutathione, GSH; ascorbic acid, AsA; etc.) and enzymatic (catalase, CAT; ascorbate peroxidase, APX; superoxide dismutase, SOD; etc.) antioxidant machinery. Both of them can work in concert to control the cascades of uncontrolled oxidation, thus protecting plant cells from oxidative damage (Asada, 2006; Miller et al., 2010; Foyer and Noctor, 2011; Suzuki et al., 2012). Additionally, it was observed that the second messengers, such as nitric oxide (NO) and cytosolic Ca^{2+}, mediate or influence GA-induced PCD (Beligni et al., 2002; De Michele et al., 2009). However, whether there exists other modulators in this process is largely unknown.

Carbon monoxide (CO), traditionally been thought of as a poisonous gas, has recently attracted increasing attention owing to its significant signaling modulatory roles (Ryter et al., 2002; Xie et al., 2008). Both in plants and animals, CO is mainly synthesized via heme oxygenase (HO; EC 1.14.99.3), which catalyzes the conversion of heme to release biliverdin IX (BV), CO and Fe^{2+} (Muramoto et al., 1999; Shekhawat and Verma, 2010). BV is subsequently reduced to bilirubin (BR) by cytosolic biliverdin reductase (Otterbein et al., 2003). Recently, the HO/CO signaling system was implicated in various plant responses against abiotic stresses (Han et al., 2008; Xie et al., 2011, 2012; Cui et al., 2012a,b; Xu et al., 2012; Jin et al., 2013a,b) and different developmental processes (Davis et al., 2001; Xuan et al., 2008, 2012; Cao et al., 2011; Lin et al., 2012; Wu et al., 2013). For example, our previous results showed that HO-1 (an inducible isoform of HO) protein level and HO activity are down-regulated in GA-treated wheat aleurone cells, whereas hydrogen peroxide (H_2O_2) content is apparently increased (Wu et al., 2011). Up-regulation of *HO-1* gene expression triggers the enhancement of CAT and APX activities or corresponding transcripts, thus resulting in a decrease of H_2O_2 production and thereafter a delay of PCD in wheat aleurone.

Similar to CO, hydrogen sulfide (H_2S) is another novel regulator of anti-inflammatory, vasorelaxant, and oxidative stress in animals (Wang, 2003). Previous results showed that H_2S release is strongly induced by oxidative stress, and H_2S-induced tolerance against oxidative stress has been also reported (Kimura, 2002; Kimura and Kimura, 2004; Calvert et al., 2010). Alongside with work in animals, increasing studies demonstrate that H_2S is involved in series of aspect of plant physiological activities in developmental and acclimatory processes, such as organogenesis (Lin et al., 2012), stomatal movement (Liu et al., 2011; Jin et al., 2013c), heat tolerance (Li et al., 2012b, 2013), salt, and drought stress adaptation (García-Mata and Lamattina, 2010; Zhang et al., 2010; Wang et al., 2012), and heavy metal stress adjustment (Zhang et al., 2008; Li et al., 2012a).

It was demonstrated that cystathionine β-synthase (CBS, EC 4.2.1.22) and cystathionine γ-lyase (CSE, EC 4.4.1.1) are two H_2S-producing enzymes in mammalian tissues (Wang, 2003). Recently, specific desulfhydrases, such as L-cysteine desulfhydrase (LCD, EC 4.4.1.1) and D-cysteine desulfhydrase (DCD, EC 4.4.1.15), have also been functionally characterized in plants (García-Mata and Lamattina, 2013; Lisjak et al., 2013). Hereinto, LCD, a homolog of mammalian CSE, acts as the most important enzyme responsible for generating H_2S in plants (Riemenschneider et al., 2005; Álvarez et al., 2010; Xie et al., 2013). Despite the roles of H_2S acting as a critical signaling molecule throughout the life cycle of plants, its physiological role in aleurone PCD and corresponding signal transduction pathway have not been fully understood.

In this study, sodium hydrosulfide (NaHS), a H_2S donor frequently used in plant and animal researches (Wang, 2003; García-Mata and Lamattina, 2010; Chen et al., 2013; Lisjak et al., 2013), was adopted to understand the physiological significance of H_2S in GA-triggered PCD in wheat aleurone cells. Two complementary approaches were simultaneously performed. First, we particular emphasized on the temporal signatures of H_2S metabolism and GSH pool, and both were decreased during GA-induced PCD process. These phenomena were just the opposite to those of ABA-treated samples. Second, the GA-treated aleurone layers were exogenously incubated with NaHS, GSH, hemin (an inducer of HO-1), and the by-products of HO-1, such as CO, BR, and Fe^{2+}, with or without a H_2S scavenger hypotaurine (HT; Ortega et al., 2008; García-Mata and Lamattina, 2010; Li et al., 2012a; Lin et al., 2012), a selective inhibitor of GSH biosynthesis L-buthionine-sulfoximine (BSO; Cui et al., 2011), or a specific HO-1 inhibitor zinc protoporphyrin IX (ZnPP; Noriega et al., 2004; Cui et al., 2012a). Combined with pharmacological and biochemical approaches, phenotypes of the PCD, H_2S metabolism, GSH pool, as well as the expression of wheat *HO-1* transcript (Xu et al., 2011) and its protein level were determined and compared. Therefore, we extended our former observations, showing that endogenous H_2S is a newly identified signaling component in the delay of GA-induced PCD by the manipulation of GSH homeostasis and HO-1 expression.

Materials and methods

Preparation for CO aqueous solution

CO gas was prepared by heating formic acid (HCOOH) with concentrated sulphuric acid (H_2SO_4) according to the reaction H_2SO_4 (l) + HCOOH (l) → CO (g) + H_2SO_4 (l) + H_2O (l). In this experiment, CO-saturated aqueous solution was freshly produced by bubbling CO gas gently through a glass tube into 50 ml of 5 mM $CaCl_2$ in a narrow neck flask for at least 20 min, a period of time long enough to saturate the solution with CO. Then, the saturated stock solution (100% saturation) was diluted immediately with 5 mM $CaCl_2$ solution to the required concentrations.

Chemicals

All chemicals were obtained from Sigma unless stated otherwise. GA and ABA were used at 50 μM. NaHS was used at concentrations of 0.1, 1.0, 10.0, and 100.0 μM, respectively. Hemin was used at 10.0 μM. Na_2SO_4, $NaHSO_4$, Na_2SO_3, $NaHSO_3$, Na_2S, and NaCl were used at 1.0 μM, respectively. The fluorescent probes, fluorescein diacetate (FDA) and *N*-(3-triethylammoniumpropyl)-4-(6-[4-(diethylamino) phenyl] hexatrienyl) pyridinium dibromide (FM 4-64), were purchased from ICN Biomedicals Inc. and Invitrogen (Molecular Probes), respectively. HT, a scavenger of H_2S, was used at 200 μM. A selective inhibitor of GSH biosynthesis, BSO, was used at 100 μM. GSH was used at 100 μM. ZnPP, a specific inhibitor of HO-1, was used at 100 μM. BR, $FeSO_4$ (Fe^{2+}), and CO were used at 1.0 μM, 1.0 μM, and 1.0% saturated aqueous solution, respectively.

Preparation of wheat aleurone layers

Aleurone layers were obtained from de-embryonated wheat (*Triticum aestivum* 'Yangmai 13') grains according to a previous method (Mrva et al., 2006; Wu et al., 2011). The de-embryonated half-grains were surface-sterilized in 1% sodium hypochlorite solution for 15 min, and washed extensively with sterile water four times. Then, the de-embryonated half-grains were imbibed in sterile water at 25 °C for 2 d. Aleurone layers were isolated by gently scraping away the starchy endosperm with a metal knife under sterile conditions.

Measurement of LCD activity

LCD activity was measured according to a previous method with some modifications (Riemenschneider et al., 2005; Xie et al.,