Biochemical and Biophysical Research Communications xxx (2016) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Cyclic ADP-ribose and IP3 mediate abscisic acid-induced isoflavone accumulation in soybean sprouts

Caifeng Jiao, Runqiang Yang, Zhenxin Gu*

College of Food Science and Technology, Nanjing Agricultural University, Nanjing, Jiangsu 210095, People's Republic of China

ARTICLE INFO

Article history:
Received 17 September 2016
Accepted 20 September 2016
Available online xxx

Keywords:
ABA-cADPR-Ca^{2+}
ABA-IP3-Ca^{2+}
Signaling pathway
Soybean sprouts
UV-B-induced isoflavone

ABSTRACT

In this study, the roles of ABA-cADPR-Ca^{2+} and ABA-IP3-Ca^{2+} signaling pathways in UV-B-induced isoflavone accumulation in soybean sprouts were investigated. Results showed that abscisic acid (ABA) up regulated cyclic ADP-ribose (cADPR) and inositol 1,4,5-trisphosphate (IP3) levels in soybean sprouts under UV-B radiation. Furthermore, cADPR and IP3, as second messengers of UV-B-triggered ABA, induced isoflavone accumulation by up-regulating proteins and genes expression and activity of isoflavone biosynthetic-enzymes (chalcone synthase, CHS; isoflavone synthase, IFS). After Ca^{2+} was chelated by EGTA, isoflavone content decreased. Overall, ABA-induced cADPR and IP3 up regulated isoflavone accumulation which was mediated by Ca^{2+} signaling via enhancing the expression of proteins and genes participating in isoflavone biosynthesis in soybean sprouts under UV-B radiation.

1. Introduction

Isoflavones, an important group of flavonoids, belong to a family of polyphenols and are synthesized predominantly in legumes. Enrichment of isoflavones has been investigated in recent decades as a result of their potential role in plants. These include playing an important role as signal molecules for the induction of nod genes, serving as precursors for the production of major phytoalexins during plant-microbe interactions, and inhibiting pathogen attack [1].

Accumulations of many secondary metabolites in plants are part of the results of defense responses to environmental stresses. Such secondary metabolite accumulation was mediated by endogenous signaling systems [2]. Our preliminary studies showed that both of UV-B radiation and ABA treatments stimulated endogenous ABA and isoflavone synthesis. The supply of UV-B radiation and exogenous ABA together caused a greater increase in endogenous ABA and isoflavone content. These data suggested that isoflavone accumulation of soybean sprouts was activated by UV-B radiation with ABA acting downstream in the signaling pathway (Supplementary Fig. S1). Nevertheless, signal transduction of ABA usually needs involvement of downstream signaling molecules.

Signal transduction pathway triggered by ABA usually involves activation of ADPRC, leading to an increase of the intracellular concentration of cADPR [3]. The amounts of cADPR in *Arabidopsis thaliana* plants increased in response to ABA treatment and before ABA-induced gene expression [4]. In addition, the activity of the enzyme responsible for cADPR synthesis, ADPRC, was rapidly induced by ABA in *Arabidopsis thaliana* plants. Furthermore, in transgenic *Arabidopsis thaliana* plants, induced expression of the *ADPRC* gene resulted in an increase of ADPRC activity and cADPR levels, as well as elevated expression of ABA-responsive genes. These results suggested that ADPRC was an early ABA-signaling event and an increase of cADPR played an important role in downstream molecular and physiological ABA responses [5]. Durner et al. [6] suggested that defense gene (*PAL* and *PR-1*) induction in tobacco by cADPR, and isoflavones played important roles in plant defense against environmental stress. Whether ADPRC/cADPR system involves in ABA mediating isoflavones accumulation in soybean sprouts under UV-B radiation needs to be deeply investigated.

Apart from ADPRC/cADPR system, the phosphatidylinositol phosphate (PtdInsP) signaling pathway is also involved in many crucial cellular functions in plants. In this pathway, signals stimulate hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2) into the second messenger IP3 via PI-PLC [7]. IP3 turnover has been shown to increase in guard-cell protoplasts of *Vicia faba* on stimulation with ABA [8]. Map-based cloning revealed that *FRY1*

Abbreviations: CHS, chalcone synthase; IFS, isoflavone synthase; ABA, abscisic acid; cADPR, cyclic ADP-ribose; ADPRC, ADP-ribosyl cyclase; IP3, inositol 1,4,5-trisphosphate; PI-PLC, phosphoinositide-specific phospholipase C.

* Corresponding author.
E-mail address: guzx@njau.edu.cn (Z. Gu).

http://dx.doi.org/10.1016/j.bbrc.2016.09.104
0006-291X/

encodes an inositol polyphosphate 1-phosphatase, which functions in the catabolism of IP3. Upon ABA treatment, *FRY1* mutant plants accumulated more IP3 than wild-type plants. These results provide the first genetic evidence indicating that phosphoinositols mediate ABA and stress signal transduction in plants and their turnover is critical for attenuating ABA and stress signals [9]. Furthermore, transgenic tobacco plants with considerably reduced levels of PI-PLC in their guard cells were partially able to regulate there stomatal apertures in response to ABA. These data implied the presence of a functional PI-PLC-based signal transduction pathway in plants that is involved in the response to ABA stimuli [10]. Both of IP3 and isoflavones accumulation are the results of stress stimuli. Nevertheless, relatively little is known about a causal relationship between PI-PLC/IP3 signaling pathway and ABA-induced isoflavones accumulation under UV-B radiation.

cADPR and IP3 are the universal and potent intracellular calcium mobilizer. Microinjection of cADPR into guard cells caused reductions in turgor which preceded by the increases in free Ca^{2+} level in the cytosol [11]. cADPR synthesized via ADPRC is a second messenger responsible for initiating the Ca^{2+} increases and subsequent occurrence Ca^{2+}-dependent phosphorylation and dephosphorylation during ABA signal transduction [4]. ABA could also induce guard cell Ca^{2+} mobilization through activating PI-PLC-dependent IP3 production [12]. Accordingly, *AtPLC1* would be the sole Ca^{2+} mobilizing mediator at endogenous ABA concentrations resting below a drought-specific threshold value that should approximate 30 μM [13]. These data suggested roles for ADPRC/cADPR and PI-PLC/IP3 systems in the generation of ABA-induced oscillations in $[Ca^{2+}]_{cyt}$. The functional effects induced by ABA are usually mediated by an increase of the intracellular calcium concentration ($[Ca^{2+}]_i$) [14]. Millisecond UV-B pulses caused an immediate rise of cytosolic calcium and lasting for more than 20 min. Increased calcium levels correlated with the subsequent stimulation of the expression of *CHS*, a isoflavonoid biosynthesis gene, indicating that the increase of calcium level might induce isoflavone accumulation. The mechanisms of Ca^{2+}-dependent cADPR and IP3 signaling pathways mediating ABA-induced isoflavone accumulation in soybean sprouts under UV-B radiation remain to be elucidated.

The objectives of this study are to investigate whether IP3 and cADPR act in the Ca^{2+}-dependent ABA signaling pathways mediating ABA-induced isoflavone accumulation in soybean sprouts under UV-B radiation, scientifically explore relevant enzymatic and molecular mechanisms.

2. Materials and methods

2.1. Plant material, growth conditions and experimental design

The washed soybean seeds (*Glycine max* L. cv *Yunhe*) were soaked with distilled water at 30 °C for 8 h, and then spread evenly in a sprouter. Temperature for germination was set as 30 °C.

For UV-B treatment, during germination, a 15 W UV-B light bulb (central wavelength 313 nm; radiation intensity 40 μW cm^{-2}) was placed at a distance of 1 ft above the sproutor in the chamber. The following chemical compounds have been used in treating soybean sprouts. All chemical compounds were purchased from Sigma-Aldrich (St. Louis, MO 63103, USA). Nicotimide as inhibitor of cADPR biosynthesis, neomycin as inhibitor of IP3 biosynthesis, EGTA as intracellular Ca^{2+} chelator. These regents were given the final concentrations of 10 μM ABA, 1 mM nicotimide, 100 μM neomycin and 0.4 mM EGTA. Controls were sprayed with distilled water only. Soybean seeds were exposed to these regent or UV-B radiation plus chemical regent for 6 days. Sprouts were sprayed with these regents three times daily (at 8:00, 16:00 and 24:00).

Table 1
Sequence-specific primers used in the this study.

Gene	Primer name	Primer sequences (5′ → 3′)
CHS	Sense	GCTTGTTGTCTGTTCTGAG
	Ant-sense	CACCTTCACTGTCTGGAG
IFS	Sense	TGGAAGTTCGTGAGGAAG
	Ant-sense	ATGGAGATGGTGCTGTTG
EF1b	Sense	CCACTGCTGAAGAAGATGATGATG
	Ant-sense	AAGGACAGAAGACTTGCCACTC

The sampled soybean sprouts used for isoflavone content determination were lyophilized using a Labconco freeze-dryer. Dried sprouts were ground to fine powder, which then was stored at −20 °C. For the other assays, the sampled fresh sprouts were immediately frozen and stored with liquid nitrogen at −80 °C.

2.2. Isoflavone analysis

The ground soybean sample was accurately weighed for 0.20 g and extracted with 6 mL of 80% methanol by ultrasonic-assisted method at 40 °C for 30 min, and then centrifuged.

The supernatant was collected. Isoflavone content was analyzed by high performance liquid chromatography (HPLC) as described by Jiao et al. [15].

2.3. Assay of key enzymes activity related to isoflavone biosynthesis

CHS activity was measured by enzyme-linked immunoassay using the CHS assay system kit (GE Healthcare) as described in the manufacturer's instructions.

IFS activity was measured according to the method of Kochs et al. [16] Activity of IFS was determined by integration of the peak areas of genistein and naringenin.

2.4. Measurement of cADPR content

Freshly harvested tissue was rapidly frozen in liquid nitrogen. Frozen tissue was pulverized into a fined powder with liquid nitrogen. The frozen powder was extracted in 0.8 vol of ice-cold 0.6 M perchloric acid and incubated on ice for 20 min. Extraction, treatment with hydrolytic enzymes and purification of cADPR was as described by Graeff and Lee [17] except that cADPR was further purified by chromatography according to Da Silva et al. [18] RIA assays were performed as described by Graeff et al. [19] using antibodies against cADPR (Chemicon International, Inc., Temecula, CA, USA).

2.5. Assay of ADPRC activity

Freshly harvested tissue was rapidly frozen in liquid nitrogen. Frozen tissue was pulverized into a fined powder with liquid nitrogen. To measure ADPRC activity, the ground powder was re-suspended in buffer containing 40 mM HEPES (pH 7.0), 100 mM NaCl, 5 mM $MgCl_2$, 4% glycerol, 0.05% NP-40, 10 mM NaF, 20 mM β-glycerolphosphate (freshly supplement with 2 mM sodium vanadate), 5 mM DTT, 1 mM PMSF, 30 nM okadaic acid, and complete EDTA-free protease inhibitors (Roche, PaloAlto, CA, USA). Re-suspended material was sonicated for 20 s and centrifuged at 12,000 g for 10 min. The resulting supernatant was then centrifuged at 100,000 g for 1 h. The membrane pellet was re-suspended in 15% glycerol, 0.02% TritonX-100 and divided into aliquots. ADPRC activity was also determined by HPLC analysis [18].

Please cite this article in press as: C. Jiao, et al., Cyclic ADP-ribose and IP3 mediate abscisic acid-induced isoflavone accumulation in soybean sprouts, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.09.104