Journal of Plant Physiology 168 (2011) 2161–2168

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.de/jplph

Gibberellins negatively regulate light-induced nitrate reductase activity in *Arabidopsis* seedlings

Yongqiang Zhang [1], Zhongjuan Liu [1], Rongzhi Liu, Liguang Wang, Yurong Bi *

School of Life Sciences, Lanzhou University, Lanzhou, Gansu 730000, People's Republic of China

ARTICLE INFO

Article history:
Received 28 February 2011
Received in revised form 11 July 2011
Accepted 13 July 2011

Keywords:
Arabidopsis
Gibberellins
Light
Nitrate reductase
Sucrose

SUMMARY

In the present study, the role of phytohormone gibberellins (GAs) on regulating the nitrate reductase (NR) activity was tested in *Arabidopsis* seedlings. The NR activity in light-grown Col-0 seedlings was reduced by exogenous GA_3 (an active form of GAs), but enhanced by exogenous paclobutrazol (PAC, a gibberellin biosynthesis inhibitor), suggesting that GAs negatively regulate the NR activity in light-grown seedlings. Light is known to influence the NR activity through both photosynthesis and phytochromes. When etiolated seedlings were transferred to white or red light, both exogenously applied GA_3 and PAC were found to function on the NR activity only in the presence of sucrose, implying that GAs are not involved in light signaling-induced but negatively regulate photoproducts-induced NR activity. NR is regulated by light mainly at two levels: transcript level and post-translational level. Our reverse transcription (RT)-PCR assays showed that GAs did not affect the transcript levels of *NIA1* and *NIA2*, two genes that encode NR proteins. But the divalent cations (especially Mg^{2+}) were required for GAs negative regulation of NR activity, in view of the importance of divalent cations during the process of post-translational regulation of NR activity, which indicates that GAs very likely regulate the NR activity at the post-translational level. In the following dark–light shift analyses, GAs were found to accelerate dark-induced decrease, but retard light-induced increase of the NR activity. Furthermore, it was observed that application of GA_3 or PAC could impair diurnal variation of the NR activity. These results collectively indicate that GAs play a negative role during light regulation of NR activity in nature.

Introduction

Nitrogen is the most abundant and essential nutrient for plants and frequently limits plant growth and development (Crawford, 1995). Nitrate is the predominant form of soil nitrogen available to plants. After being taken up by plants, nitrate will be reduced to ammonia for incorporation into amino acids (Cheng et al., 1992; Crawford, 1995). The pathway of nitrate assimilation has already been clearly determined. The first step committed in the nitrate assimilation in plants is the reduction of nitrate to nitrite by nitrate reductase (NR, EC 1.6.6.1) through catalyzing the transfer of two electrons from nicotinamide-adenine dinucleotide phosphate [NAD(P)H] to nitrite, which is further reduced in the chloroplast by reduced ferredoxin and nitrite reductase (NiR, EC 1.7.7.1) (Crawford, 1995; Lillo et al., 2004; Du et al., 2008). Generally, the activity of NiR in plants is much higher than that of NR, which enables plants to avoid nitrite accumulation to a toxic level. Thus, the NR is the rate-limiting step in the nitrate assimilation pathway (Pilgrim et al., 1993; Du et al., 2008). In *Arabidopsis*, two different genes *NIA1* and *NIA2* encoding NR have been identified many years ago (Wilkinson and Crawford, 1993).

NR is regulated by several environmental stimuli. Of them, light plays a very important role as an external signal for regulation of the gene expression and activity of NR (Pilgrim et al., 1993; Lillo and Appenroth, 2001). In the past decades, it has been clear that NR is regulated by light mainly at two different levels: (1) at the transcriptional level, regulating *NIA1* and *NIA2* expressions, which represents a rough regulation on a time scale of hours or days; (2) at the post-translational level, which represents fine-tuning on a time scale of minutes or hours (Lillo and Appenroth, 2001). Both photosynthetically active light and light acting through phytochrome are known to influence the NR (Cheng et al., 1992; Appenroth et al., 2000). It has been known that phytochrome-mediated effects of light on NR are evident in etiolated leaves, and in green leaves the roles of photoreceptor would be generally overtaken by chlorophyll (acting via photosynthesis) (Appenroth et al., 2000). So, the regulation of NR by phytochrome has been studied mainly in etiolated tissues. Although the effects of phytochrome on NR have been determined, the downstream components of phytochrome

Abbreviations: GA, gibberellin; HY5, long hypocotyl 5; HYH, HY5 homolog; NR, nitrate reductase; PAC, Paclobutrazol.

* Corresponding author. Tel.: +86 931 8912560; fax: +86 931 8912561.
E-mail address: yrbi@lzu.edu.cn (Y. Bi).

[1] These authors contributed equally to this work.

0176-1617/$ – see front matter © 2011 Elsevier GmbH. All rights reserved.
doi:10.1016/j.jplph.2011.07.004

in this signaling pathway still remain less clear. Recently, two bZIP (basic leucine zipper) transcription factors long hypocotyl 5 (HY5) and HY5 homolog (HYH) were identified to be downstream components of phytochrome functioning as positive factors of NR regulation (Jonassen et al., 2008, 2009), and *NIA2* transcript may be positively regulated by HY5 in *Arabidopsis* leaves (Lee et al., 2007; Jonassen et al., 2009). Nevertheless, the effect of light on NR of green plant seedlings might be quite different from that of the etiolated seedlings. For example, only white light, but not red, far-red or blue light, was able to stimulate significant accumulation of NR mRNA in green barley seedlings (Melzer et al., 1989). Cheng et al. (1992) proved that sucrose, as the product of photosynthesis, could mimic the light induction of NR gene transcription, and made the conclusion that light regulation of NR genes in green plant seedlings was likely to be mediated indirectly through sugars produced by photosynthesis. But to date, how sucrose regulates NR expression is still unclear (Jonassen et al., 2008).

NR modification at the post-translational level is achieved through the reversible protein phosphorylation that provides a more rapid regulation of NR activity (Kaiser et al., 2002). Generally, NR is inactivated in darkness by phosphorylation at a special serine residue (serine 534 in *Arabidopsis*), and activated by dephosphorylation in light (Lillo et al., 2004). Post-translational regulation of NR by light is probably achieved through the effects of photosynthesis (photosynthetic products), which has been concluded from experiments using green leaves in which the effects of photosynthetic active light on NR have been studied (Kaiser and Huber, 2001; Lillo et al., 2004). Notably, it was found that the photosynthetically active light but not the phytochrome system was involved in the post-translational regulation of NR by phosphorylation and the 14-3-3s binding (Appenroth et al., 2000).

In addition to light, phytohormones may be another important factor affecting NR activity and expression in plants. The inductions of NR activity and *NIA1* gene expression by cytokinin were observed in *Arabidopsis* (Yu et al., 1998) and barley (Lu et al., 1990, 1992). In contrast with cytokinin, abscisic acid plays a negative role in induction of the NR activity and gene expression (Lu et al., 1992). The gibberellins (GAs) have effects on the regulation of NR activity as well as gene expressions in *Arabidopsis* (Bouton et al., 2002) and affect the NR activity together with kinetin in tobacco leaves (Roth-Bejerano and Lips, 1970). GAs are a group of diterpenoids that are biosynthesized from geranylgeranyl diphosphate, and among more than 100 GAs identified from plants, only a few of them, such as GA_1, GA_3, GA_4 and GA_7, have been shown to be physiologically active in controlling seed germination, leaf expansion, stem elongation and flowering (Yamaguchi, 2008). More interestingly, an increasing number of reports indicate that light and GAs could be regulated interactively. For example, light was found to affect GAs biosynthesis or signaling in both *Arabidopsis* seeds (Oh et al., 2006) and seedlings (Achard et al., 2007; Zhao et al., 2007), while the role of the GAs in light-mediated plant growth such as hypocotyl elongation was also found recently (Alabadí et al., 2004, 2008; Achard et al., 2007). Although Bouton et al. (2002) made some observations on GAs regulating NR in *Arabidopsis*, the detailed role of GAs in light-induced NR activity is still unclear. Hence, here we attempt to examine the effect of GAs on light-induced NR activity in *Arabidopsis* seedlings.

Materials and methods

Plant materials and growth conditions

Unless otherwise specified, the *Arabidopsis thaliana* accession Col-0 was used in our experiments. Seeds were surface-sterilized with 20% (v/v) bleach solution for about 13 min, and then rinsed with sterile water and sown on agar medium in Petri dishes on half-strength Murashige and Skoog (1/2MS) salts containing 0.8% (w/v) agar without any sugars. Agar plates were kept at 4 °C in the dark for 3 days, and then transferred to a growth chamber maintained at 23 °C under continuous white light (about 75 $\mu mol\ m^{-2}\ s^{-1}$) for 6 days before treatment. For GA_3, GA_{4+7} (active forms of GAs) or PAC (Paclobutrazol, a GA biosynthesis inhibitor) treatment, *Arabidopsis* seedlings were transferred to fresh 1/2MS media containing various concentrations of these chemicals.

Determination of NR activity

About 30 mg seedlings were homogenized with 1 mL of 0.1 M HEPES–KOH buffer solution (pH 7.5) containing 3% (w/v) polyvinylpolypyrrolidone (PVPP), 1 mM ethylenediamine tetraacetic acid (EDTA) and 7 mM cysteine. The homogenate was centrifuged for 10 min at 10 000 g at 4 °C, and the supernatant was used for NR activity analysis according to the methods described by several previous reports (Lea et al., 2006; Du et al., 2008; Jonassen et al., 2008). NR activity was calculated as $\mu mol\ NO_2^-$ produced per hour and per gram of fresh weight ($\mu mol\ NO_2^-\ g^{-1}\ FW\ h^{-1}$).

Semi-quantitative reverse transcription (RT)-PCR and quantitative RT-PCR (qRT-PCR) assays

Seedlings were harvested in liquid nitrogen, ground, and RNA was extracted using TRIzol reagent (Invitrogen). For semi-quantitative RT-PCR, the complementary DNA (cDNA) was synthesized using a random hexamer primer and Moloney murine leukemia virus (M-MuLV) reverse transcriptase (Fermentas) at 42 °C for 60 min. Equal amounts of cDNA, estimated using reactions with *ACTIN2* primers, were used as templates for PCR amplification with the following protocol: 5 min denaturation at 94 °C followed by indicated cycles with each cycle composed of 94 °C for 30 s, 53, 55 and 56 °C, for genes *ACTIN2*, *NIA1*, and *NIA2*, respectively for 30 s, 72 °C for 1 min, and 10 min at 72 °C in the end. PCR products were visualized by electrophoresis on 1.2% (w/v) agarose gels containing ethidium bromide. For qRT-PCR, first-strand cDNA was synthesized from 1 μg of total RNA using PrimeScript RT reagent Kit with gDNA Eraser (TaKaRa). PCR reactions were performed in triplicate using the CFX96 Real Time System (Bio-Rad) with SYBR Premix Ex Taq II Kit (TaKaRa), according to the procedure described by the manufacture. The raw data were analysed with CFX Manager Software (version 1.1), and expression was normalized to *ACTIN2* to minimize variation in cDNA template levels. Relative expression levels were calculated using the comparative threshold (Ct value) method. Fold changes ($2^{-\Delta\Delta Ct}$) were expressed relative to the control. Mean values were obtained from three biological replicates. Primer sequences used for semi-quantitative RT-PCR and qRT-PCR can be found in Table S1.

Results

GAs negatively regulate light-induced NR activity

To test the possible effects of GAs on regulation of light-induced NR activity in *Arabidopsis*, 6-day-old white light-grown and dark-grown Col-0 seedlings were treated with various concentrations of GA_3, GA_{4+7}, or PAC, and after an additional 3 days still retained in the same corresponding conditions, the NR activities of the seedlings were determined. For light-grown seedlings, as shown in Fig. 1, with applied GA_3 concentrations increased from 0 to 1000 μM, the NR activities decreased from 1.69 to 0.54 $\mu mol\ NO_2^-\ g^{-1}\ FW\ h^{-1}$, and similarly, for GA_{4+7} treatment, with the concentrations increased to 1000 μM, the NR activity decreased to 0.22 $\mu mol\ NO_2^-\ g^{-1}\ FW\ h^{-1}$. In contrast, with the