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Short communication

Expression of succinate dehydrogenase and fumarase genes in maize leaves is mediated by cryptochrome

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ABSTRACT

Blue light inhibits succinate dehydrogenase and fumarase enzyme activity and gene expression in green leaves of maize (*Zea mays* L.). Irradiation of maize plants by blue light resulted in the transient decrease of transcripts of genes *Sdh1-2* and *Sdh2-3* encoding correspondingly the flavoprotein and iron-sulfur protein subunits of succinate dehydrogenase, and of *Fum1* encoding the mitochondrial form of fumarase. The blue light effect was probably mediated by transcription factors COP1 and HY5, with the expression of the latter increased upon blue light treatment. This was accompanied by a decrease in the expression of COP1, presumably involved in proteasome degradation of HY5. It was also demonstrated that calcium ions do not participate in this process.

1. Introduction

Inhibition of plant respiration in the light is an important mechanism of metabolic regulation in green tissues of plants during active photosynthesis (Igamberdiev et al., 2014; Gardeström and Igamberdiev, 2016). It proceeds via inhibition of the pyruvate dehydrogenase complex (Gemel and Randall, 1992), regulation of NAD- and NADP-dependent isocitrate dehydrogenases (Igamberdiev and Gardeström, 2003) and inhibition of succinate dehydrogenase (SDH) and fumarase, which can take place at the level of regulation of enzyme activity (Daloso et al., 2015) and via modulation of gene expression caused by phytochrome A and mediated by calcium ions (Popov et al., 2010; Eprintsev et al., 2013, 2016).

Another possible mechanism of regulation of respiration includes the effects of blue light absorbed by cryptochromes (Cashmore et al., 1999; Lopez et al., 2012; Fortunato et al., 2015; Fox et al., 2015), which was initially observed for SDH in Arabidopsis using the cryptochrome mutants (Eprintsev et al., 2015). The effect of blue light is mediated by the positive photomorphogenic regulator HY5 (Oyama et al., 1997; Osterlund et al., 2000a) and negative photomorphogenic regulators ubiquitin ligases COP1 and COP9 (Osterlund et al., 1999). In darkness, COP1 is transferred to the nucleus where it interacts with HY5 and undergoes degradation (von Arnim and Deng, 1994; Osterlund et al., 2000b). Blue light modulates the subcellular localization of COP1 by directing it to the cytosol (Osterlund et al., 2000b).

HY5 is a transcription factor localized constitutively in the nucleus (Ang et al., 1998). It binds to the G-box of light-induced promoters and ensures optimal expression of the corresponding genes (Yang et al., 2005; Zhang et al., 2011). Degradation of HY5 in darkness provides the mechanism by which its activity and the HY5-mediated gene expression can be regulated in the light (Chattopadhyay et al., 1998; Zhang et al., 2011).

Following our previous study (Eprintsev et al., 2015) where we established the involvement of cryptochrome in SDH expression in Arabidopsis, we studied the mechanism of cryptochrome-dependent regulation of SDH and fumarase genes in maize. We report new data revealing possible mechanism of intracellular transduction of photoreceptor signal to the nucleus leading to the alterations in expression of the genes encoding the SDH subunits and two forms of fumarase.

2. Materials and methods

Green leaves of 14-days-old maize (*Zea mays* L., cv. Voronezhskaya 76) plants grown hydroponically at 22 °C and 12 h light period were used in the experiments. Plants were placed in the dark chamber for 24 h and illuminated for 15 min by blue light of the intensity 0.044 W m^{-2} generated by light diodes with irradiation wavelength 465–470 nm (Proton, Russia).

SDH (EC 1.3.99.1) and fumarase (EC 4.2.1.2) activities were measured on the UV–vis spectrophotometer T70 + (PG Instruments Ltd, UK). The unit of enzymatic activity was defined as the amount of enzyme forming 1 µmol of product per minute at 25 °C. SDH was measured by a decrease of absorption at 600 nm, caused by reduction of the artificial electron acceptor dichlorophenolindophenol (DCPIP), using

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the extinction coefficient of oxidized DCPIP 21 $mM^{-1}cm^{-1}$ (Cooper and Beevers, 1969). The reaction medium contained 50 mM potassium phosphate buffer, pH 7.8, 1 mM NaN_3, 10 mM succinate, 0.1 mM phenazine metasulphate, and 0.008 mM DCPIP. Fumarase activity was measured by an increase in optical density at 240 nm due to the formation of the double bond in fumarate molecule. The assay medium contained 50 mM potassium phosphate, pH 7.0, 50 mM malate and 5 mM $MgCl_2$. The extinction coefficient of fumarate 2.44 $mM^{-1}\,cm^{-1}$ was used (Moore and Proudlove, 1983).

The nuclear fraction was isolated according to Lee and Lin (2005). The amount of free calcium was measured spectrophotometrically using the color reaction with murexide in the presence of glycerol (Scarpa, 1972).

The total RNA was isolated from maize leaves by the guanidinum thiocyanate–phenol–chloroform extraction according to Chomczynski and Sacchi (1987). The polymerase chain reaction with gene-specific primers was performed using the reagents AmpliSens (Helicon, Russia) (Eprintsev et al., 2017). The primers for PCR analysis had the following nucleotide sequences: for *Sdh1-2* forward – 5′-CGAATGGGTCATTGC CAACT-3′; reverse – 5′-ACCTTTGAAAGGGTACAAAA-3′, for *Sdh2-3*: forward – 5'-GAGAGGCTACAGGCAATAACTGAG-3', reverse – 5'-GGATTTTGACTTGCATGGGATTG-3', for *Cop1*: forward – 5′-TCTGCG TCCACAGATAGCAC-3′, reverse – 5′-GTCTGGCGATCCAAATCTGT-3′, for *Hy5*: forward – 5′-ATTGAGTTGCAGGGATGGAG-3′, reverse – 5′-CCCTCTGTAGCCTGTTGAGC-3′, for *Fum1*: forward, 5′-GATTACTTC GATCATTGAGGT-3′; reverse, 5′-ACCAGAACTCGCGGATGTGGC-3′; for *Fum2*: forward, 5′-ACAAACTTGCCATTCGTCACC-3′; reverse, 5′-TGGT TCATTCTCAGGCAGAGA-3′.

The polymerase chain reaction was performed in the amplifier Tercik (DNA-Technology, Moscow, Russia). The real-time polymerase chain reaction (RT-PCR) was performed on the LightCycler 96 (Roche, Switzerland) using SYBR Green I as a dye. The parameters of amplification included an initial denaturation at 95 °C for 5 min followed by 40 cycles: 20 s at 95 °C, 30 s at 58 °C, 40 s at 72 °C, and, finally, 4 min at 72 °C. The matrix quantity was normalized relatively to the gene of the elongation factor *Ef-1ά* (Nicot et al., 2005).

Determination of relative expression of the studied genes was performed by the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001).

All experiments were repeated three to four times and statistically evaluated. The data on the figures are means of three biological repeats ± SD. The statistically significant differences at $P < 0.05$ are discussed.

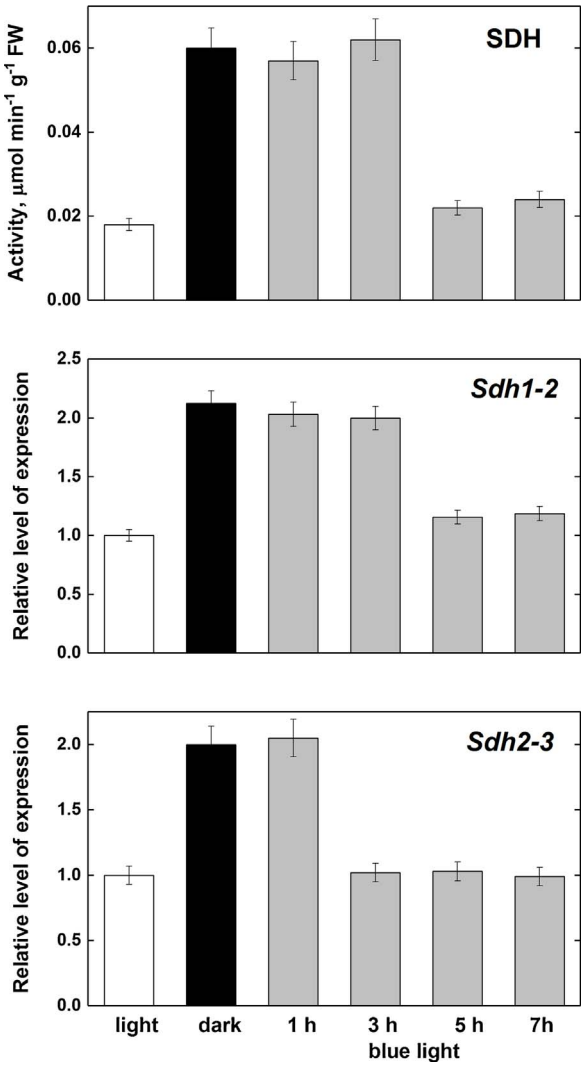

Fig. 1. The effects of light regime on succinate dehydrogenase activity and expression of genes *Sdh1* and *Sdh2*. White bars: plants exposed to the light of intensity 25 W m^{-2}; black bars – plants exposed to darkness for 24 h; grey bars – plants exposed to 15 min irradiation with blue light and transferred to darkness for 1, 3, 5 and 7 h.

3. Results

SDH activity was more than three times higher in darkness than in the light (Fig. 1). Irradiation by blue light did not result in activity changes during the first 3 h, but from 5 h the activity decreased by 2–3 times and approached the level of SDH activity in the light. The data on SDH activity corresponded to the pattern of expression of *Sdh1-2* which was two times higher in darkness than in the light and decreased to the same level as in the light from 5 h after irradiation by blue light. Expression of *Sdh2-3* revealed a similar pattern with the earlier effect observed at 3 h after blue light irradiation (Fig. 1).

Fumarase activity revealed a similar pattern as SDH (Fig. 2). We studied the effect of blue light for this enzyme and expression of its genes only 3 h after irradiation. The activity was three times higher in darkness than in the light and after irradiation by blue light decreased to even much lower values than in the continuous daylight. Expression of *Fum1* encoding the mitochondrial form of fumarase revealed the same pattern as the activity of fumarase. On the contrary, expression of *Fum2* encoding the cytosolic form of fumarase, was lower in darkness but irradiation by blue light decreased its level even further to the extremely low values (Fig. 2).

To study possible roles of COP1 and HY5 factors in the transduction of the cryptochrome signal, the level of transcription of corresponding genes was determined (Fig. 3). In darkness, the transcript level of *Cop1* gene increased significantly, while the level of *Hy5* gene was much lower than in the light. After irradiation by blue light the decrease in *Cop1* expression was observed and its level dropped already after 1 h of irradiation below the level observed in the light and remained at the same low level in the next hours. Opposite to the expression of *Cop1*, the level of *Hy5* increased after blue light irradiation and peaked at 3–5 h after the treatment, although remaining lower than in the continuous daylight (Fig. 3). The level of calcium in the nuclei of maize cells was twice higher in darkness than in the light, however, no significant changes were observed after the treatment with blue light (Fig. 3).

4. Discussion

The activities of SDH and fumarase were markedly inhibited by light (Figs. 1 and 2) which corresponds to the earlier findings (Popov et al., 2010; Eprintsev et al., 2013, 2015, 2016). It was shown earlier that this inhibition was caused by red light via the action of phytochrome A and mediated by calcium (Eprintsev et al., 2013, 2016). However, the preliminary data (Eprintsev et al., 2015) revealed that blue light was also efficient in suppressing SDH activity and that cryptochromes may