SHORT COMMUNICATION

Signal transduction controlling the blue- and red-light mediated gene expression of S-adenosylmethionine decarboxylase in Pharbitis nil

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Abstract

The signal transduction processes involved in the regulation of SAMDC gene expression by blue and red light were examined using pharmacological inhibitors of signalling pathways. Calcium and calmodulin positively regulated SAMDC gene expression in red light, whereas in blue light they regulated negatively. These results indicate that calcium homeostasis is involved in both red and blue light induction of SAMDC expression. Both signal transduction pathways also require new protein synthesis.

Key words: Blue-light receptor, Pharbitis nil, phytochrome, S-adenosylmethionine decarboxylase, signal transduction.

Introduction

Polyamines are small, positively charged aliphatic amines found in all living organisms. They are involved in a variety of processes in plants, including the regulation of DNA replication, transcription of genes, cell division, organ development, fruit ripening, and leaf senescence (Kumar \textit{et al.}, 1997). Their biosynthetic pathway is relatively well established. Putrescine is produced directly from ornithine via ornithine decarboxylase (ODC; EC 4.1.1.17) or indirectly from arginine via arginine decarboxylase (ADC; EC 4.1.1.19). Spermidine and spermine are formed by the subsequent addition of an aminopropyl moiety onto putrescine and spermidine. The aminopropyl moiety results from the decarboxylation of S-adenosylmethionine (SAM) by S-adenosylmethionine decarboxylase (SAMDC; EC 4.1.1.50). SAMDC is considered to be the rate-limiting enzyme for the synthesis of spermidine and spermine (Kumar \textit{et al.}, 1997; Tiburcio \textit{et al.}, 1997).

It was reported that SAMDC activity in leaves of \textit{Pharbitis nil} increased dramatically after illumination (Hirasawa and Shimada, 1994). The photoresponse of SAMDC activity is regulated primarily at a transcriptional level (Yoshida \textit{et al.}, 1998). Seedling responses to light are mediated by at least three classes of regulatory photoreceptors: phytochromes which respond to red and far-red light, blue/UV-A photoreceptors that are specific for blue and UV-A light and UV-B photoreceptors (Arnim and Deng, 1996). A previous study suggested that both phytochrome and blue light photoreceptor-mediated pathways were involved in the light regulation of SAMDC gene in \textit{Pharbitis nil} (Yoshida \textit{et al.}, 1999).

In recent years, signal transduction pathways controlling photoresponses in plants has been demonstrated. Three distinct signal transduction cascades involving cGMP and/or calcium were found to control PhyA responses downstream of the G protein (Bowler \textit{et al.}, 1994a). The cGMP-dependent pathway regulates the expression of chalcone synthase (CHS) and anthocyanin biosynthesis. The calcium-dependent pathway regulates the expression of chlorophyll \textit{a/b}-binding protein (\textit{CAB}) and partial chloroplast development. A third pathway, requiring both cGMP and calcium, is utilized to activate ferredoxin NADP\(^{+}\) oxidoreductase (\textit{FNR}) expression and to produce fully activated chloroplasts. These pathways are able to cross-regulate each other.

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cGMP and Ca\textsuperscript{2+}/CaM were shown to exhibit interpathway regulation wherein downstream regulators of one pathway can negatively regulate one another (Bowler et al., 1994b). The phototransduction pathways by blue/UV-A and UV-B receptors are largely uncharacterized. A role for calcium and calmodulin in controlling the UV-mediated induction of \textit{CHS} gene expression has been proposed (Christie and Jenkins, 1996; Frohnmeyer et al., 1998).

In this study, a pharmacological approach was used to examine signal transduction processes concerned with the induction of \textit{SAMDC} expression by red and blue light. It is shown that proteins synthesized \textit{de novo} are required for the \textit{SAMDC} gene expression in both phytochrome- and blue light photoreceptor-mediated pathways. It is proposed that, whereas calcium and calmodulin are positive regulators for the phytochrome control of \textit{SAMDC} gene expression, they act as negative regulators for blue light-mediated \textit{SAMDC} gene induction.

### Materials and methods

#### Plant material and light treatments

Seeds of \textit{Pharbitis nil} (strain Violet) were germinated at 25 \textdegree C for 1 d. The germinated seeds were then grown for 4 d at 22 \textdegree C under continuous light from fluorescent lamps (FL40S-PG; Panasonic, Osaka, Japan) at a photosynthetic photon flux density of 100 \textmu mol m\textsuperscript{-2} s\textsuperscript{-1} at the top of the plants as described in our previous study (Yoshida et al., 1999). The seedlings were transferred to 16 h darkness (21 \textdegree C and 30–40% RH) prior to 45 min of light exposure. For light treatments, red light was supplied by red fluorescent lamps (FL40S-R-F; Panasonic) which emit light between 610–710 nm at a fluence rate of 60 \textmu mol m\textsuperscript{-2} s\textsuperscript{-1} and blue light was supplied by blue fluorescent lamps (FL40S-B-F; Panasonic) which emit light between 390–540 nm at a fluence rate of 60 \textmu mol m\textsuperscript{-2} s\textsuperscript{-1}.

#### Treatments with chemical antagonists

Nifedipine (Calbiochem, Darmstadt, Germany) and K252a (Calbiochem) were dissolved in dimethyl sulphoxide (DMSO). Cycloheximide (Sigma, St Louis, MO), trifluoperazine (Sigma), W-7 (Calbiochem), and okadaic acid (Sigma) were dissolved in distilled water. Each was diluted to 1/1000, 1/2500 or 1/5000, respectively, with distilled water when applied to the seedlings. Five-day-old seedlings of \textit{Pharbitis nil} grown under continuous light were placed for 15 h in the dark. The roots of seedlings were cut in darkness and the cut seedlings were incubated in the solutions of inhibitors for an additional 1 h in the dark. For controls, the cut seedlings were treated with equivalent amounts of distilled water or 0.1\% DMSO. These treatments did not affect transcript levels.

#### RNA extraction and Northern blot analysis

Leaves of \textit{Pharbitis nil} were harvested, immediately frozen in liquid N\textsubscript{2} and stored at −80 \textdegree C. Total RNA was isolated from the leaves using TRIsol reagent (Gibco-BRL, Egggenstein, Germany) according to the manufacturer’s instructions. Northern blot analysis was carried out using \textit{Pharbitis}

\textit{SAMDC} DNA probe (GenBank accession No. AF018285) as previously described (Yoshida et al., 1998). The blot was stripped with boiling for 5 min in 0.01\% SDS and subsequently rehybridized with \textit{Pharbitis} rDNA probe (D89869) as a control. For quantification, Northern blots were scanned with Bio-imaging Analyser System BAS 1000 (Fuji Photo Film Co., Ltd., Tokyo, Japan). The relative abundance of \textit{SAMDC} transcripts were normalized to the amount of rRNA loaded per lane.

### Results and discussion

The signal transduction processes involved in regulation of \textit{SAMDC} gene expression by blue and red light were examined using pharmacological inhibitors of signalling pathways. To determine whether protein synthesis is required for blue- and red-light induction of \textit{SAMDC}, the effect of cycloheximide on \textit{SAMDC} gene expression was examined. The induction of the \textit{SAMDC} gene after blue and red light exposure was largely inhibited by cycloheximide (Fig. 1). It seems very likely that at least one new protein that is essential for \textit{SAMDC} expression in both blue light- and phytochrome-mediated pathways. The induction of \textit{CHS} by UV-B and UV-A blue light in \textit{Arabidopsis} cells has been shown to require cytoplasmic protein synthesis. It is suggested that one or more components, such as a transcription factor, are essential for the stimulation of expression and are synthesized after illumination (Christie and Jenkins, 1996). It is possible that the protein synthesized \textit{de novo} is the transcription factor. Wang et al. have isolated the gene for a protein designated CCA1 which can bind to the promoter of an \textit{Arabidopsis} light-harvesting chlorophyll \textit{a/b} protein gene, Lhcb1*3 (Wang et al., 1997). The CCA1 protein acts as a specific activator of \textit{Lhcb1*3} transcription in response to red light. The expression of \textit{CCA1} is transiently induced by phytochrome and oscillates with the circadian clock (Wang and Tobin, 1998). In addition, CCA1 protein was shown to be expressed rhythmically and also to be involved in the circadian rhythm of \textit{Lhcb} gene expression. They also suggested that CCA1 is closely associated with the circadian clock in \textit{Arabidopsis}. A previous study showed that the circadian clock controls the transcription of \textit{SAMDC} gene (Yoshida et al., 1999). Expression of \textit{SAMDC} might be controlled by the transcription factor such as CCA1. The identification of the new protein(s) required for \textit{SAMDC} expression is a priority.

Calcium is involved in a variety of plant responses, including phytochrome, blue/UV-A- and UV-B-regulated gene expression (Bowler et al., 1994b; Christie and Jenkins, 1996; Frohnmeyer et al., 1998). Calcium regulates the activities of target proteins directly or via calcium-binding proteins such as calmodulin. In this study, the calcium channel blocker nifedipine and the calmodulin antagonists trifluoperazine and W-7 were used to demonstrate the involvement of calcium and calmodulin in blue and red light signalling pathways regulating \textit{SAMDC}
induction in *Pharbitis nil*. Nifedipine, trifluoperazine and W-7 superinduced SAMDC gene in blue light, whereas they were inhibitory in red light (Fig. 2). These results indicate that, whereas calcium and calmodulin may be negative regulators of blue light photoreceptor-mediated SAMDC gene regulation, they may be positive regulators of phytochrome-mediated regulation. Recent studies have demonstrated that calcium homeostasis in a cytosolic pool has been implicated in the signalling processes of both phytochromes and cryptochromes (Frohnmeyer et al., 1998; Long and Jenkins, 1998). The increase in calcium and calmodulin above an optimal level may inhibit SAMDC expression by blue light, and the decrease in calcium and calmodulin below an optimal level may inhibit SAMDC expression by red light. One possibility is that calcium homeostasis may be required for the photoinduction of SAMDC gene. Red or blue light transiently increases cytosolic Ca$^{2+}$ (Shacklock et al., 1992; Baum et al., 1999) and cytosolic Ca$^{2+}$ exhibits circadian rhythmicity (Johnson et al., 1995; Wood et al., 2001). A previous report showed that SAMDC mRNA accumulation is controlled by the endogenous circadian clock (Yoshida et al., 1999). The change in levels of cytosolic Ca$^{2+}$ concentration might contribute to SAMDC gene expression. Alternatively, Guo et al. have identified SUB1, an Arabidopsis Ca$^{2+}$-binding protein (Guo et al., 2001). SUB1 was reported to be a component of a cryptochrome signalling pathway and a negative regulator of photomorphogenesis and gene expression. The other possibility is that Ca$^{2+}$-binding protein, such as SUB1, may be involved in the photoinduction of SAMDC gene. Further research is needed to examine these possibilities.

Recent studies have demonstrated that protein phosphorylation and dephosphorylation are important mechanisms of phototransduction pathways (Chory and Wu, 2001; Yamagata et al., 2001). It has been suggested that the phosphorylation and dephosphorylation of specific proteins, including phytochrome, is involved in the phytochrome-mediated light-signal transduction pathway (Choi et al., 1999; Fankhauser et al., 1999). Some blue light responses have been shown to involve protein phosphorylation (Huala et al., 1997). In this study, the effects of protein kinase and phosphatase inhibitors...
on the blue- and red-light regulation of SAMDC in Pharbitis nil were examined. Incubation of the seedlings with protein phosphatase inhibitor okadaic acid prevented SAMDC gene expression by red light, whereas it did not by blue light (Fig. 3). These results indicate that components in the phytochrome signalling pathway regulating SAMDC are different from that in the blue light signalling pathway. However, the protein kinase inhibitor had no inhibitory effect on the induction of SAMDC by both blue and red light (Fig. 3). There is a possibility that the protein kinase inhibitor added externally may not have reached the site where protein kinase actually exists. Further new experimental tools or systems are needed to examine this.

Several recent reports have shown that phyB transported into the nucleus in the Pfr form recruits transcription factors such as PIF3 and induces transcription of light-regulated genes, such as CCA1 and LHY, after interacting with the light regulatory cis-acting elements of the target genes (Martinez-Garcia et al., 2000; Nagy and Schäfer, 2000). In a previous study, it was suggested that phyB is involved in the light induction of SAMDC expression and that a circadian clock controls the amplitude of the response (Yoshida et al., 1999). In addition, this study showed that proteins synthesized de novo are required for the SAMDC gene expression in the phytochrome-mediated pathway. There is a possibility that a transcription factor, such as CCA1 which is closely associated with the circadian clock, may be synthesized de novo by phyB-mediated red light input and control the gene expression of SAMDC. Further study is required to find out the transcription factor involved in light regulation of SAMDC gene expression in Pharbitis nil.

References


