

Effects of light and phytohormones on the expression of cucumber ferrochelatases

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Introduction

Chlorophylls (Chls) and hemes are major tetrapyrrole compounds in photosynthetic organisms, playing essential roles in such processes as photosynthesis and respiration. In higher plants, these compounds are synthesized from glutamyl-tRNA via 5-aminolevulinic acid (ALA), a linear five-carbon molecule. The branch point of the biosynthetic pathway of heme and Chls is protoporphyrin IX, a closed-macrocycle without chelated ions. Ferrochelatase catalyzes the chelation of ferrous ion into the protoporphyrin IX to form protoheme, while Mg-chelatase catalyzes the insertion of magnesium ion to subsequent formation of Chls.

ALA is formed by the two-step conversion from glutamyl-tRNA; glutamyl-tRNA is first reduced to glutamate 1-semialdehyde (GSA) in an NADPH-dependent reaction by glutamyl-tRNA reductase, and then GSA is transaminated by GSA aminotransferase to form ALA (Kannangara et al. 1998). Among these steps, reduction of glutamyl-tRNA is proposed to be a regulatory point for the synthesis of tetrapyrroles (Beale 1999). Two cDNAs encoding glutamyl-tRNA reductase (*hemA1* and *hemA2*) were isolated from cucumber (Tanaka et al. 1996). The expression of *hemA1* was light-inducible and its transcript was detected primarily in photosynthetic tissues, whereas the expression of *hemA2* was not light-responsive and its transcript was detected predominantly in non-photosynthetic tissues. Previous work has also shown that the level of *hemA1* mRNA increased by cytokinin, namely benzyladenine, which stimulates total Chl biosynthesis in cucumber (Masuda et al. 1995).

Meanwhile, two cDNAs of ferrochelatase (*CsFeC1* and *CsFeC2*) were isolated from cucumber (Miyamoto et al. 1994, Suzuki et al. submitted). Recently we showed that the mRNA of *CsFeC1* primarily accumulated in non-photosynthetic tissues and its level was not changed by light, and that of *CsFeC2* accumulated in all tissues and its level was increased by light in cotyledons (Suzuki et al. 2000, submitted). Furthermore, we found that the expressions of both *hemA2* and *CsFeC1* were greatly induced by cycloheximide in cotyledons (Suzuki et al. submitted). These similar expression profiles of isoforms of glutamyl-tRNA reductase and ferrochelatase suggest that tetrapyrrole biosynthetic pathways in photosynthetic and non-photosynthetic tissues are distinctly controlled by coordinated regulation of isoforms of these two key enzymes. Furthermore, as both heme and Chls are synthesized in chloroplasts, the flux of the protoporphyrin IX into both pathways must be tightly regulated by ferrochelatase and Mg-chelatase.

To gain further insight into this regulation, in this paper, we examined the effect of light and phytohormones on the expression of cucumber ferrochelatases.

Materials and Methods

Plant materials - Etiolated cucumber seedlings (*Cucumis sativus* L. cv Aonagajibai) were grown on moist gauze at 26°C in a growth chamber in darkness for 4 days. For the experiments on the circadian regulation, cucumber was grown under 12-h light/12-h dark cycles. The cotyledons of 5-day old plants were collected every 3 h for 24 h.

Treatment with phytohormones - Cotyledons excised from the 4-day old etiolated seedlings were incubated on the filter paper moistened with 3.5 ml of various phytohormone solutions (100 µM) in a petri dish. As the reference of solvent effects, water for abscisic acid (ABA), ACC and BA, and ethanol for GA₃, 2,4-dichlorophenoxy acetic acid (2,4-D), MeJA and salicylic acid (SA) were used.

Northern blotting analysis - 20 µg of total cucumber RNA were used for Northern blotting. A fragment of *Arabidopsis* 25S rRNA cDNA was used as a probe for detection of cucumber rRNA. Conserved regions (398 - 1648 nt) of *CsFeC2* cDNA and (398 - 1639 nt) of *CsFeC1* cDNA were used as probes.

Western blotting analysis - Antibodies specific for *CsFeC1* and *CsFeC2* were prepared and used for Western blot analyses.

Results and Discussion

Effect of illumination on mRNA expression of *CsFeC2* - The levels of *CsFeC2* mRNA in etiolated cotyledons gradually increased in response to illumination (Fig. 1A). In cotyledons, the increase of *CsFeC2* protein was also observed after 48-h illumination (Fig. 2B). The increase of mRNA and protein levels by illumination was also observed in hypocotyls but not in root (data not shown). As reported previously, the levels of mRNA and protein of *CsFeC1* were not changed by illumination (Suzuki et al. 2000). We should note that the light-dependent induction profile of the *CsFeC2* mRNA accumulation in cotyledons was similar to that of *hemA1*, which was also induced within 3 h and reached a high steady state level up to 24 h (Tanaka et al. 1996).

Our previous Western blotting and *in vitro* import experiments demonstrated that *CsFeC2* mainly localized in thylakoid membranes of chloroplasts (Suzuki et al. submitted). Since *CsFeC2* and Mg-chelatase co-exist in chloroplast, the change of transcript level of *CsFeC2* by light and circadian rhythm must be important for the regulation of chlorophyll and heme synthesis. In fact, previous work in this laboratory has already shown that the expression of Mg-chelatase subunit (CHLH) is tightly regulated by light and diurnal oscillation (Nakayama et al. 1998). Thus, the level of *CsFeC2* mRNA was investigated throughout a diurnal cycle by harvesting cotyledons at 3-h intervals during 12-h light / 12-h dark cycle. In contrast to the Mg-chelatase, the level of the *CsFeC2* transcript did not change significantly during the cycle (data not shown). This result is not consistent with the result reported by Papenbrock et al. (1999). The disagreement might be due to young cotyledons (5-day old) used in the present study as compared with 4-week old tobacco leaves. Further study is needed to clarify this discrepancy.

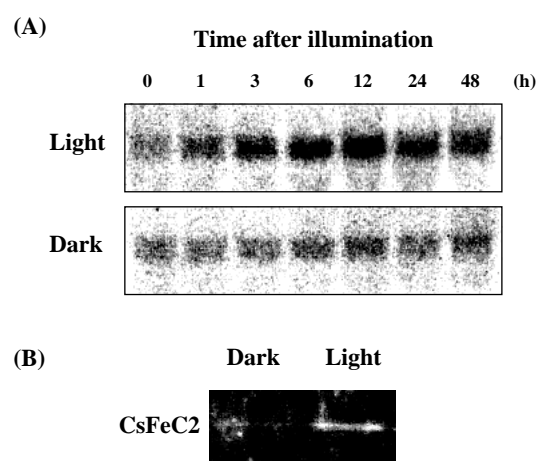


Fig 1. Time course of light induced-expression of cucumber *CsFeC2*. Etiolated cucumber cotyledons were illuminated with white light. (A), mRNA levels; (B), protein levels.

Effects of phytohormones and related compounds on CsFeC2 expression - Then, we examined the effects of phytohormones on the mRNA expressions of *CsFeC1* and *CsFeC2* in etiolated cotyledons. Our previous study revealed that in cotyledons *CsFeC1* mRNA was not expressed irrespective of illumination (Suzuki et al. 2000) without cycloheximide treatment (Suzuki et al. submitted). Treatment (24 h) with phytohormones tested in this study neither induced *CsFeC1* mRNA (data not shown). Among phytohormones, ACC, BA, GA₃ and MeJA increased the levels of the *CsFeC2* mRNA in etiolated cotyledons (Fig. 2A). BA, a synthetic cytokinin, had the most pronounced effect on the increase in the *CsFeC2* mRNA level. The effective concentration of BA was 1 μ M up to 100 μ M (data not shown). The BA-induced increase in the *CsFeC2* mRNA was observed after 1-h treatment of 100 μ M BA, reached maximum at 6 h, and then gradually decreased up to 48 h after treatment (Fig. 2B). Western blot analysis showed that the protein level of CsFeC2 was also increased by BA (Fig. 2C). Again this induction profile of the *CsFeC2* mRNA was similar to that of *hemA1*, which showed transient increase of the mRNA level after BA-treatment (Masuda et al. 1995).

The present data support the hypothesis that two of respective isoforms of glutamyl-tRNA reductase and ferrochelatase are coordinately controlled to regulate independent tetrapyrrole biosynthetic pathways in photosynthetic and non-photosynthetic tissues. Since light and BA have generally stimulative effects on chloroplast proteins such as protochlorophyllide oxidoreductase in tetrapyrrole biosynthesis (Kuroda et al. 1996) and monogalactosyl diacylglycerol synthase in galactolipid biosynthesis (Ohta et al. 1995), we can not exclude the possibility that light- and BA-induced expressions of *hemA1* and *CsFeC2* are caused by general effect during chloroplast development, rather than specific phenomena to these two isoforms. Further analysis of expression of these genes is necessary for better understanding of their regulation.

In summary, we propose that as well as Mg-chelatase, the Chl and heme biosynthetic pathways in chloroplasts of photosynthetic tissues are regulated by coordinate expression of *hemA1* and *CsFeC2*, which is inducible light and BA. On the other hand, the regulation of heme biosynthesis in plastids of non-photosynthetic tissues is dependent on *hemA2* and *CsFeC1*, those are not light-responsive and sensitive to cycloheximide.

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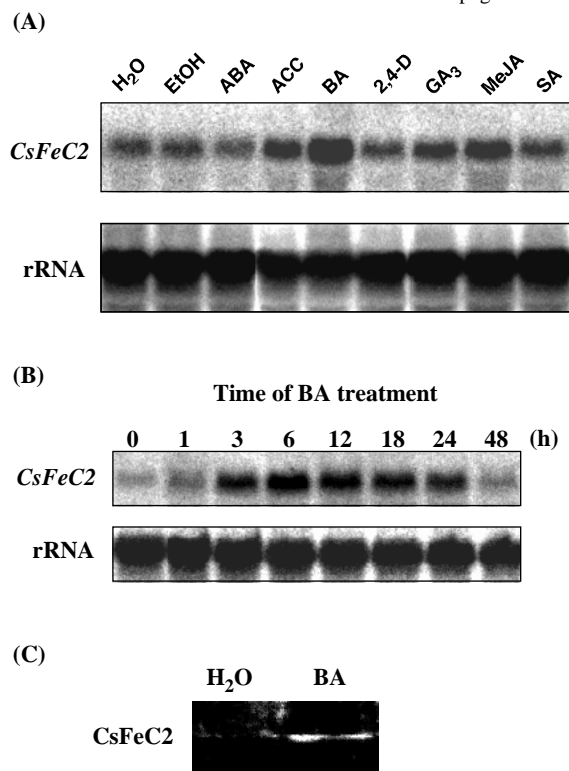


Fig. 2. Effects of treatment with phytohormones on expression of cucumber *CsFeC2*. Etiolated excised cucumber cotyledons were treated with various phytohormones of 100 μ M and mRNA levels were examined in (A). (B), Time course of *CsFeC2* mRNA expression after BA treatment; (C), Increase of *CsFeC2* by 24-h treatment of BA.

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