Volume 239, number 2, 199–202 FEB 06454 November 1988

Action spectra for photogene expression in etiolated pea seedlings

Yukiko Sasaki, Kazuichi Yoshida[+] and Atsushi Takimoto[+]

Department of Food Science and Technology and [+] *Department of Agricultural Biology, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan*

Received 5 September 1988

Action spectra (responsivity spectra) for the transcript accumulation of four photogenes, *rbc*S, *rbc*L, *Cab* and *psa*B, had a major peak in the red region (660 nm). Violet (400 nm) and green (510 nm) light also induced these transcripts, but their effects differed with the gene. The effect of red light was reversed by later exposure to far-red light, but the effects of violet and green light were not. Non-reversible photoreactions in addition to the phytochrome-mediated photoreversible reaction seemed to be involved in the expression of these photogenes.

Action spectrum; Light induction; Photogene expression; Phytochrome; Violet light; Green light; (Pea seedling)

1. INTRODUCTION

Light stimulates the synthesis of several proteins related to photosynthesis in plant leaves. In greening pea leaves, the induction of ribulose bisphosphate carboxylase/oxygenase (RuBisCO) and chlorophyll *a/b*-binding protein (Cab) is caused by an increase in mRNA [1–3], which occurs after a brief exposure to red light. One of the photoreceptors involved in this reaction seems to be phytochrome because the effect of red light is partly reversed by later exposure to far-red light [3–9]. However, the effects of brief exposure to other wavelengths of monochromatic light have not yet been examined in detail. Here, we studied the action spectra (spectral dependence) for the expression of some photogenes in etiolated pea seedlings. The photogenes examined were the nuclear-encoded small subunit gene of RuBisCO (*rbc*S), the nuclear-encoded Cab gene (*Cab*), the chloroplast-encoded large subunit gene of RuBisCO (*rbc*L) and the chloroplast-encoded PS I A2 subunit gene (*psa*B).

Correspondence address: Y. Sasaki, Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan

2. MATERIALS AND METHODS

2.1. *Plant growth and light treatment*

Pea seedlings (*Pisum sativum* var. Alaska) grown at 20°C in complete darkness for 5 days were irradiated for 5 min with monochromatic light with a narrow bandwidth at the indicated wavelength (10 $\mu mol \cdot m^{-2} \cdot s^{-1}$) by use of the Okazaki large spectrograph at the National Institute for Basic Biology, Okazaki, Japan [10]. The seedlings were then returned to darkness. After 2 days in the dark, 10 apical buds were harvested and immediately frozen in liquid nitrogen. All manipulations were done by Noct-vision (NEC) under the illumination of long-wavelength far-red light (>850 nm; a Toshiba IR-D cut-off filter was used).

2.2. *Preparation of RNA and measurement of transcript abundance*

Total RNA was extracted from the frozen apical buds by the SDS-phenol/chloroform method and precipitated with ethanol. The ratio of RNA to DNA in the precipitates of the crude extract [11] was almost the same in all preparations. The total RNA was purified four times by LiCl precipitation to remove contaminating DNA. From 10 apical buds, about 0.8 mg total RNA was obtained. To check the mRNA activity of each preparation, its template activity in an in vitro protein-synthesizing system from wheat germ was measured [12]. Almost the same activity was found in each RNA preparation. The total RNA was glyoxylated, electrophoresed, and transferred to a nylon membrane (BNNG, Pall). For quantitative analysis, 1.0, 0.5, or 0.25 μg of the RNA was dot-blotted on a nylon membrane (BNRG, Pall). The membrane was baked, boiled for 5 min in 20 mM Tris-HCl buffer (pH 8.0), prehybridized, and hybridized with nick-translated ^{32}P-labelled probes as described elsewhere [12]. The probes used were: *rbc*S,

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

the 0.75-kbp *Cla*I fragment of pGR 407 from pea cDNA [13]; *Cab*, the 0.6-kbp *Bam*HI fragment of pea cDNA [14]; *rbc*L, the 1.2-kbp *Bam*HI fragment of the large subunit of RuBisCO from tobacco chloroplast [15]; and *psa*B, the 2.0-kbp *Eco*RI-*Xho*I fragment of PS I A2 subunit genc from tobacco [15]. The specific activity of the probes was about $0.5–1.7 \times 10^8$ cpm/μg. The probe concentration in hybridization buffer was about 0.06–0.18 μg/ml. After hybridization, the membrane was washed and autoradiographed for an appropriate time [12]. The mRNA levels were measured by tracing the X-ray film with a densitometer (CS-930, Shimadzu). After confirmation that the data followed a straight line in 0–1.0 μg RNA, and subtraction of the value of the sample without irradiation (control), the value at 0.5 μg RNA was expressed relative to the value at 660 nm. The value attained with 660-nm light was taken as 1.

3. RESULTS AND DISCUSSION

Etiolated 5-day-old seedlings were briefly irradiated (5 min) with monochromatic light at various wavelengths (290–750 nm), and the effect of the irradiation on the transcript abundance in the pea buds was examined 2 days later. The fresh weight of pea buds was affected by the brief irradiation, but the amount of total RNA per cell did not change, because the ratio of RNA to DNA in each crude extract was constant. Template activity of each unit of RNA preparation for the in vitro protein-synthesizing system was affected slightly (not shown). We can use the transcript abundance in a fixed amount of total RNA as an estimate reflecting the levels in a cell.

The time course of the appearance of the effect of red light differed among transcripts [16], but the transcript abundance examined here increased almost linearly, at least up to day 2 after exposure to the light pulse (fig.1). The basal level of *rbc*S transcript in darkness was lower than the levels of *Cab*, *rbc*L, and *psa*B, and two days later, the *rbc*S transcript increased about 15-fold over that without irradiation, while the *Cab*, *rbc*L, and *psa*B transcripts increased 5–7-fold. The mRNA abundance for the mitochondrial ATP synthase β-subunit gene (*atp* 2-1) encoded in nuclear DNA [17], which is not stimulated by light, was at the constitutive level and used as control mRNA (fig.1).

Northern blots of the total RNA showed that *rbc*S, *Cab*, and *rbc*L probes hybridized with the corresponding mature mRNA size, and that the *psa*B probe hybridized with the co-transcribed mRNA size [18], indicating that these probes were specific to the respective transcripts (fig.2). The

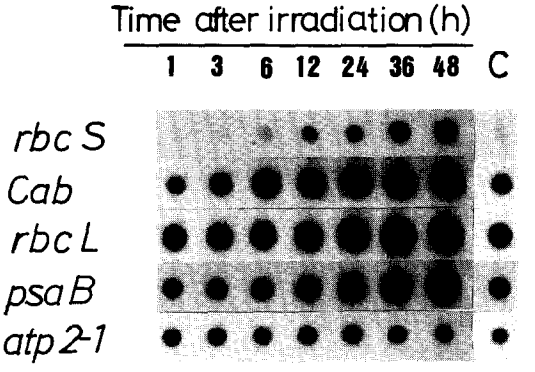

Fig.1. Dot-blots of total RNA showing time course of transcript induction in darkness after irradiation with red light. Etiolated 5-day-old seedlings were exposed to red light (660 nm, 10 μmol·m^{-2}·s^{-1}) for 5 min and returned to darkness. After the indicated time, the total RNA was extracted, dot-blotted, and hybridized as described in section 2. 1 μg of the RNA was dotted. The control (C) was the RNA from etiolated 7-day-old seedlings without irradiation with red light. The specific activity was 0.6×10^8 cpm/μg for *rbc*S and *Cab*, 0.7×10^8 cpm/μg for *rbc*L and *psa*B, and 1.7×10^8 cpm/μg for *atp* 2-1. The probe concentration in hybridization buffer was 0.06 μg/ml. The exposure time was 67 h for *rbc*S, *Cab*, *rbc*L and *atp* 2-1, and 21 h for *psa*B.

brief irradiation at 400 or 660 nm affected the abundance of the four transcripts variously, but not that of *atp* 2-1 (fig.2). The relative amount of each transcript in total RNA was measured by quantitative dot-blot hybridization by use of these probes. The effects of various wavelengths on transcript abundance was summarized as the action spectra (fig.3).

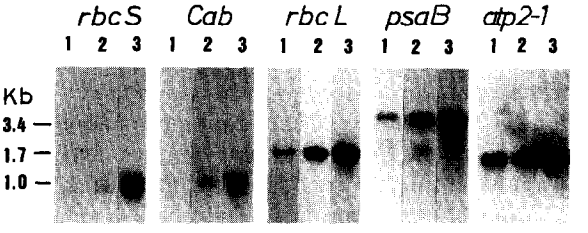

Fig.2. Northern blots of total RNA showing specificity of probes and effects of light irradiation. Etiolated 5-day-old seedlings were exposed to 400- or 660-nm light (10 μmol·m^{-2}·s^{-1}) for 5 min and returned to darkness. Two days later, total RNA was extracted, glyoxalated, electrophoresed, and treated as described in section 2. The membrane was exposed to X-ray film for the appropriate time. 6 μg of RNA was electrophoresed in each lane. Lane 1, no light control; lane 2, 400-nm light; lane 3, 660-nm light.

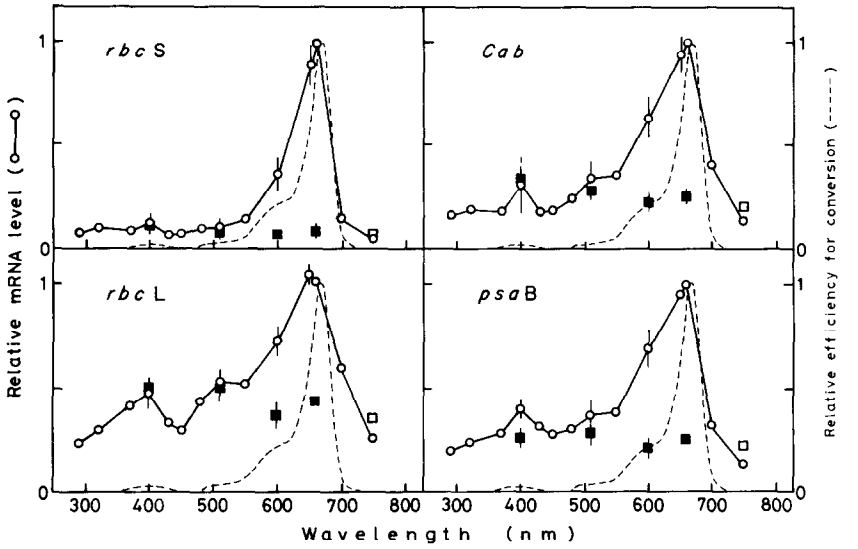

Fig.3. Action spectra for the expression of four photogenes. From plants treated with various wavelengths as described in the legend of fig.2, the total RNA was extracted and treated as described in section 2. The mean of three or more analyses was plotted. Action spectra for the photoconversion of Pr to Pfr [19] (– – –) are reproduced for comparison. (○) Response to the indicated wavelength (10 μmol·m^{-2}·s^{-1}, 5 min). (■) Response to far-red light (750-nm, 80 μmol·m^{-2}·s^{-1}) immediately after a light pulse of 400-, 510-, 600-, or 660-nm light (10 μmol·m^{-2}·s^{-1}, 5 min). (□) Response to far-red light given alone at the same fluence rate. Vertical bars indicate standard deviations.

In our preliminary experiments, the effects on *rbc*L transcript abundance of a 5-min exposure to monochromatic light of 450, 550, or 660 nm at 1, 10, and 50 μmol·m^{-2}·s^{-1} were examined. Light of 660 nm at 10 μmol·m^{-2}·s^{-1} was fully effective, but that of 450 or 550 nm at the same fluence rate and that of 660 nm at 1 μmol·m^{-2}·s^{-1} were less effective. Therefore, the transcript abundance for *rbc*S, *Cab*, *rbc*L, and *psa*B was measured after 5-min irradiation with monochromatic light at 10 μmol·m^{-2}·s^{-1} (fig.1).

The action spectrum (responsivity spectrum) for the *rbc*S transcript had a large peak at 660 nm, without any other large peak. This spectrum closely coincided with the action spectrum for the in vivo photoconversion of the red-absorbing form of phytochrome (Pr) to the far-red absorbing form (Pfr) [19] (fig.1), and the induction by 600- and 660-nm light was almost completely reversed by later far-red irradiation at 750 nm. This suggests that the induction of the transcript is mediated by phytochrome [3–9].

The action spectrum for the *rbc*L transcript also had a major peak at 660 nm, but with a minor peak at 400 nm (violet) and a clear shoulder at 510 nm (green). The amount induced by 400- and 510-nm light was one-half of that induced by the 660-nm light. The effects of violet and green light were similar but weaker for the *Cab* and *psa*B transcripts (fig.1).

The level of induction of *rbc*L, *Cab*, and *psa*B transcripts by 600- and 660-nm light was reversed by later exposure to far-red light (750 nm) to the level of the far-red light control, indicating that phytochrome was involved in the reaction (fig.1). On the other hand, the induction of these transcripts by 400- or 510-nm light was not reversed by a later far-red pulse, except for slight reversal of the induction of the *psa*B transcript by 400-nm light. Some non-reversible photosystem in addition to the reversible phytochrome system may be involved in the expression of these photogenes.

The level of transcript accumulation induced by violet and green light relative to that induced by red light differed with the gene, and even for the *rbc*S transcript, violet and green light were slightly effective. This suggests that the above two systems contribute to all four photogenes examined here in various degrees, depending on the gene. Note that two of the above four genes are encoded in the nucleus and the remaining two in chloroplasts.

Because each action spectrum in the region be-

tween 300 and 510 nm appears to be similar to that for photoconversion of Pr to Pfr, although at a different level, the non-reversible induction of the transcripts by violet and green light may be caused by the so-called very low fluence response (VLFR) mediated by phytochrome. In fact, Kaufmann et al. [20] reported that a very low fluence red light (below 10^{-3} μmol·m^{-2}) affected the induction of the *Cab* transcript. Detailed studies are required for the elucidation of the photosystem operating in the violet and green regions.

Acknowledgements: We thank Drs M. Sugiura, K. Shinozaki, S.M. Smith, and N.H. Chua for the gift of plasmids, Drs M. Watanabe and K. Yamamoto for help with spectroscopy, and Dr R. Matsuno for encouragement. This research was supported by grants from the Ministry of Education, Science, and Culture, and from the Ministry of Agriculture, Forestry and Fisheries, Japan. This study was carried out under the NIBB Cooperative Research Program.

REFERENCES

[1] Smith, S.M. and Ellis, R.J. (1981) J. Mol. Appl. Genet. 1, 127–137.
[2] Sasaki, Y., Ishiye, M., Sakihama, T. and Kamikubo, T. (1981) J. Biol. Chem. 256, 2316–2320.
[3] Jenkins, G.I., Hartley, M.R. and Bennett, J. (1983) Philos. Trans. R. Soc. London Ser. B 303, 419–431.
[4] Sasaki, Y., Sakihama, T., Kamikubo, T. and Shinozaki, K. (1983) Eur. J. Biochem. 133, 617–620.
[5] Thompson, W.F., Everett, M., Polans, N.O., Jorgensen, R.A. and Palmer, J.D. (1983) Planta 158, 487–500.
[6] Gallagher, T.F., Jenkins, G.I. and Ellis, R.J. (1985) FEBS Lett. 186, 241–245.
[7] Tobin, E.M. and Silverson, J. (1985) Annu. Rev. Plant Physiol. 36, 569–593.
[8] Fluhr, R. and Chua, N.H. (1986) Proc. Natl. Acad. Sci. USA 83, 2358–2362.
[9] Fluhr, R., Kuhlemeier, C., Nagy, F. and Chua, N.H. (1986) Science 232, 1106–1112.
[10] Watanabe, M., Furuya, M., Miyoshi, Y., Inoue, Y., Iwahashi, I. and Matsumoto, K. (1982) Photochem. Photobiol. 36, 491–498.
[11] Sasaki, Y., Nakamura, Y. and Matsuno, R. (1987) Plant Mol. Biol. 8, 375–382.
[12] Sasaki, Y., Tomoda, Y., Tomi, H., Kamikubo, T. and Shinozaki, K. (1985) Eur. J. Biochem. 152, 179–186.
[13] Anderson, S. and Smith, S.M. (1986) Biochem. J. 240, 709–715.
[14] Cashmore, A.R. (1984) Proc. Natl. Acad. Sci. USA 81, 2960–2964.
[15] Shinozaki, K., Ohme, M., Tanaka, M., Wakasugi, T., Hayashida, N., Matsubayashi, T., Zaita, N., Chuywongse, J., Obokata, J., Yamaguchi-Shinozaki, K., Ohto, C., Torazawa, K., Meng, B.Y., Sugita, M., Deno, H., Kamogashira, T., Yamada, K., Kusuda, J., Takaiwa, F., Kato, A., Tohdoh, N., Shimada, H. and Sugiura, M. (1986) EMBO J. 5, 2043–2049.
[16] Kaufman, L.S., Roberts, L.L., Briggs, W.R. and Thompson, W.F. (1986) Plant Physiol. 81, 1033–1038.
[17] Boutry, M. and Chua, N.H. (1985) EMBO J. 4, 2159–2165.
[18] Woodbury, N.W., Roberts, L.L., Palmer, J.D. and Thompson, W.F. (1988) Curr. Genet. 14, 75–89.
[19] Pratt, L.H. and Briggs, W.R. (1966) Plant Physiol. 41, 467–474.
[20] Kaufman, L.S., Thompson, W.F. and Briggs, W.R. (1984) Science 226, 1447–1449.