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Effects of Light on Spinach Glycolate Oxidase Gene Expression

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Abstract: Glycolate oxidase is one of the key enzymes in the pathway of photorespiration. In this study we investigated the effects of light on the expression of the spinach glycolate oxidase gene. Continuous exposure to white light resulted in a gradual increase in the steady-state level of glycolate oxidase mRNA within a time period of 2~24 h in both etiolated and dark-adapted green seedlings. A short white light pulse also increased the level of glycolate oxidase mRNA in etiolated seedlings. The mRNA level reached a maximum at 6~8 h after the pulse and decreased by 24 h after the pulse. The induction patterns of the glycolate oxidase gene by white light appeared similar to those of the rbcS gene, indicating that a common or coordinating regulatory system may be involved in the expression of the glycolate oxidase and rbcS genes. A red light pulse induced an increase in the amount of glycolate oxidase mRNA and this effect was reversed by a subsequent far-red light pulse, suggesting that the expression of the glycolate oxidase gene is regulated by phytochrome.

Key words: glycolate oxidase, photoregulation, photorespiration, rbcS, spinach.

Light regulates the expression of many plant genes at the transcriptional level. The two best-known examples are the rbcS and cab genes, which encode the small subunit of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and the chlorophyll a/b binding proteins, respectively. The effects of light on the expression of these genes have been shown to be mediated by phytochrome (Stiekema *et al.*, 1983; Silverthorne and Tobin, 1984). In addition to phytochrome, blue/UV-A photoreceptor and UV-B photoreceptor are considered to play important roles in the photocontrol of these genes (Fluhr and Chua, 1986; Wehmeyer *et al.*, 1990; Dedonder *et al.*, 1993). Presumably a complex network of signal transduction pathways is required for light-regulated gene expression in plants.

Photorespiration involves light-dependent evolution of CO_2 with the associated consumption of O_2. Although the physiological role of photorespiration is not clear yet, it is believed that photorespiration serves to recycle oxidation products generated by the oxygenase activity of Rubisco. The expression of photorespiratory genes, like photosynthetic genes, seems to be induced by light. The expression of the gene encoding glycolate oxidase, one of the key enzymes in the pathway of photorespiration, has been reported to be controlled by light in lentil (Gerdes and Kindle, 1988) and spinach (Park *et al.*, 1992). However, the photoregulatory mechanism of glycolate oxidase gene expression remains largely unknown.

In this study we examined the expression patterns of the glycolate oxidase gene by light and compared them with those of the rbcS gene in spinach. The results presented here suggest that phytochrome is involved in the light-regulated expression of the glycolate oxidase gene and that the glycolate oxidase and rbcS genes are regulated in a coordinate manner.

Materials and Methods

Plant growth

Spinach seeds were germinated for 3 days and grown hydroponically either for 7 days in the dark (10-day-old etiolated seedlings) or for 4 days under continuous white fluorescent light (7-day-old green seedlings) at ambient temperature. Green seedlings were placed in the dark for 3 days before light treatment.

Light sources

For continuous white light treatments, a fluorescent

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lamp (18 W, 1200 lumen) from Dongmyoung electrical company was used. For light pulse experiments, a Kodak CAROUSEL 4600 projector lamp (300 W) was used. Red and far-red lights were obtained using a red glass filter (center wavelength: 660 nm, bandwidth: 10 nm) and a far-red glass filter (center wavelength: 725 nm, bandwidth: 10 nm), respectively. These filters were purchased from Oriel corporation (Stanford, Connecticut, USA). Plants were placed 40 cm away from the light sources and the filters were located halfway between the plant and the light source.

Northern blot analysis

Total RNA was prepared from spinach leaves using the phenol/SDS method (De Vries *et al.*, 1988). Total RNA (20~25 μg) was electrophoresed in a 1.2% agarose/2.2 M formaldehyde gel and transferred to a nitrocellulose filter using 20X SSC as described by Sambrook *et al.* (1989). Equal loading of RNA was checked by ethidium bromide staining of the gel, or by methylene blue staining of the blots after hybridization (Sambrook *et al.*, 1989). The blots were hybridized with a glycolate oxidase cDNA (a 1.5 kb *Eco*RI fragment of pMV21) or a rbcS cDNA (a 0.8 kb *Not*I fragment of pJA300). These probes were labeled using the ECL direct nucleic acid labeling system (Amersham). pMV21 (Volokita and Somerville, 1987), which contains a spinach glycolate oxidase cDNA, was provided by Dr. Somerville at Michigan State University. pJA300 contains a spinach rbcS cDNA that was obtained by screening a spinach leaf cDNA library using a soybean rbcS cDNA as a probe (Jin *et al.*, manuscript in preparation).

Results and Discussion

Effect of continuous white light on glycolate oxidase gene expression

We examined the induction kinetics of glycolate oxidase gene expression by white light in green spinach seedlings. Seven-day-old, light-grown seedlings were dark-adapted for 3 days and then exposed to continuous white light for 2, 4, 6, 8, or 24 h. Total RNAs isolated from these samples were subjected to Northern blot hybridization and the results are shown in Fig. 1. The three-day-dark treatment did not completely abolish the steady-state levels of glycolate oxidase and rbcS mRNAs. A significant increase in the steady-state level of glycolate oxidase mRNA was observed after 4 h of illumination and the level kept increasing with irradiation time (Fig. 1A). Previous studies on the expression of the spinach glycolate oxidase gene have shown that the amount of glycolate oxidase mRNA reached a maximum after 24 h of illumination (Park *et al.*, 1992). The induction of rbcS mRNA, like glycolate oxidase mRNA, was distinct after 4 h of illumination and the mRNA level increased with irradiation time (Fig. 1B).

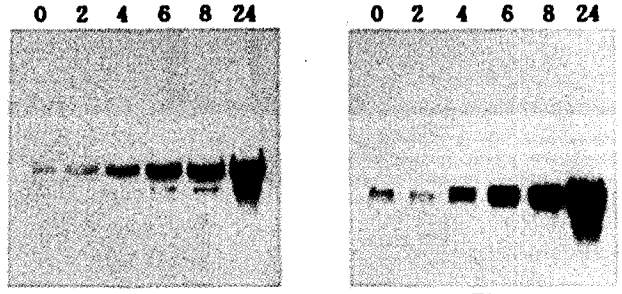

Fig. 1. Induction kinetics of glycolate oxidase (A) and rbcS (B) mRNAs by white light in green seedlings. Seven-day-old green seedlings were dark-adapted for 3 days and then exposed to continuous white light for 0, 2, 4, 6, 8, or 24 h. Total RNA (20 μg) isolated from each of these samples was analyzed by Northern blot hybridization.

The effect of continuous white light on the expression of the glycolate oxidase gene was also examined in 10-day-old etiolated seedlings. The results are shown in Fig. 2. Substantial levels of both glycolate oxidase and rbcS mRNAs were found in the etiolated seedlings, indicating that these two genes are expressed at the basal level in the dark. The amounts of the two mRNAs began to increase 2 h after light exposure and kept increasing up to 24 h. The results of Figs. 1 and 2 indicate that the response to white light occurs more rapidly in etiolated than in dark-adapted green seedlings. It is conceivable that etiolated plants possess a more sensitive light signal transduction pathway than green plants. The overall induction kinetics of the glycolate oxidase and rbcS genes by continuous white light, regardless of the developmental condition of the plant, appeared similar. This may reflect the need of cooperative action of the two gene products, that is, the accumulation of the photooxidative intermediates resulting from the activation of Rubisco by light might require the concomitant activation of photorespiratory enzymes, including glycolate oxidase.

Effect of white light pulse on glycolate oxidase gene expression

To better understand the role of light as a signal for the activation of glycolate oxidase gene, we examined the effect of a short light pulse on the expression of the gene in etiolated seedlings. Ten-day-old etiolated seedlings were exposed to 10 min of white light, placed in darkness for 2, 4, 6, 8, or 24 h, and harvested for Northern blot analysis. The time course of glycolate oxidase and rbcS mRNA accumulation is shown in Fig.

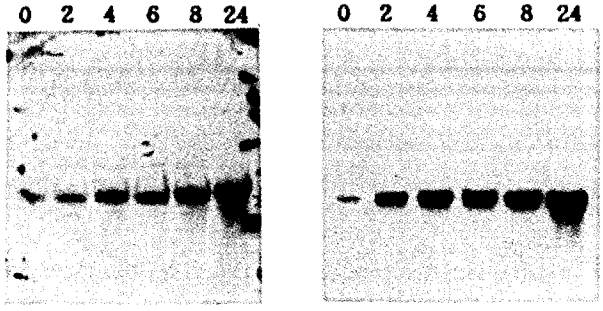

Fig. 2. Induction kinetics of glycolate oxidase (A) and rbcS (B) mRNAs by white light in etiolated seedlings. Ten-day-old dark-grown seedlings were exposed to continuous white light for 0, 2, 4, 6, 8, or 24 h. Total RNA (20 μg) isolated from each of these samples was analyzed by Northern blot hybridization.

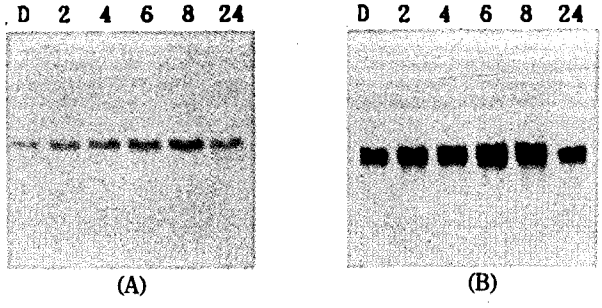

Fig. 3. Time course of glycolate oxidase (A) and rbcS (B) mRNA accumulation in response to a white light pulse. Ten-day-old dark-grown seedlings were exposed to 10 min white light pulse and placed in the dark for 2, 4, 6, 8, or 24 h (D, no light exposure). Total RNA (25 μg) isolated from each of these samples was analysis by Northern blot hybridization.

3. The mRNA levels of both genes reached a maximum at 6~8 h after the light pulse and decreased by 24 h after the pulse. These parallel induction patterns of the two genes raise the possibility that a regulatory system may operate to ensure the coordinate expression of the glycolate oxidase and rbcS genes.

The results of Figs. 2 and 3 show that the induction of glycolate oxidase gene expression by a short light pulse is weaker than that by continuous light. Presumably continuous presence of light is required for the full expression of the gene. The simplest explanation for this observation is that the activation of the glycolate oxidase gene depends on the quantity of light. It is also possible that the stability of the mRNA is enhanced in the presence of light. The stability of rbcS mRNA has been reported to be altered by exposure to light (Fritz *et al.*, 1991; Wanner and Gruissem, 1991).

Involvement of phytochrome in glycolate oxidase gene expression

To determine whether the expression of the glycolate oxidase gene is regulated by phytochrome, 10-day-old

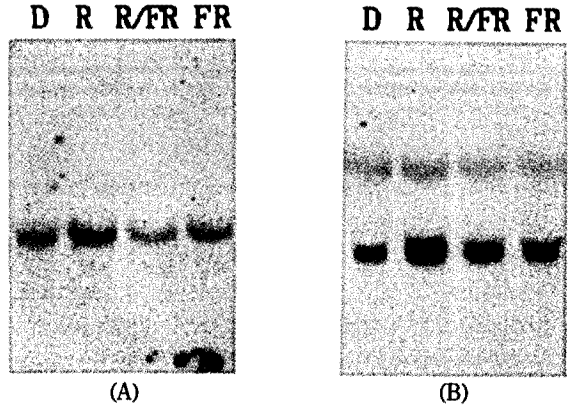

Fig. 4. Involvement of phytochrome in the activation of glycolate oxidase (A) and rbcS (B) genes. Ten-day-old dark-grown seedlings were exposed to no light (D), 10 min red light (R), 10 min red light followed by 15 min far-red light (R/FR), or 15 min far-red light (FR). Total RNA (20 μg) was isolated from each of these samples after 4 h of darkness and analyzed by Northern blot hybridization.

etiolated seedlings were exposed to 10 min of red light, 10 min of red light followed by 15 min of far-red light, or 15 min of far-red light. Total RNA was isolated from the seedlings after 4 h of darkness and analyzed by Northern blot hybridization. The results are shown in Fig. 4. The red light pulse induced a slight increase in the amount of glycolate oxidase mRNA, and this effect was reversed by the subsequent far-red light pulse (Fig. 4A), which is the operational criterion for the phytochrome effect. The far-red light pulse alone did not appear to increase the amount of glycolate oxidase mRNA (Fig. 4A). The results of Fig. 4B show that the induction of rbcS gene expression is mediated by phytochrome, as expected.

The results shown in Fig. 4 indicate that phytochrome plays a role in the expression of both the glycolate oxidase and rbcS genes. However, the magnitude of the response of the glycolate oxidase gene to a red light pulse was reproducibly smaller than that of rbcS gene. This observation could mean that the light signal transduction pathways may differ in the two gene systems. Or this may simply be due to the difference in gene copy number between the two genes. A genomic Southern hybridization experiment using a 5′ fragment of glycolate oxidase gene as a probe resulted in a single band, suggesting that the glycolate oxidase gene is a single-copy gene (unpublished data). On the other hand, all known plant rbcS genes exist in families of 4 to 13 genes (Dean *et al.*, 1989). Indeed, genomic Southern blot analysis suggested that multiple rbcS genes are present in spinach (unpublished data). It should be noted that the rbcS mRNA described in this study may represent the total mRNA species because the probe used is expected to hybridize to the highly con-

served coding sequences of the rbcS gene family. Several types of photoreceptors are known to exist in plants and the expression of many plant genes, including the rbcS (Fluhr and Chua, 1986; Dedonder *et al.*, 1993) and cab (Wehmeyer *et al.*, 1990) genes, has been shown to be regulated by interactions of various photoreceptors. It will be interesting to see if other light receptors, in addition to phytochrome, are involved in the regulation of glycolate oxidase gene expression.

A light signal is believed to affect gene expression mainly by changing the binding behaviors of trans-acting factors to the promoter regions of target genes. The promoter regions of rbcS genes have been extensively studied and many interacting trans-acting factors have been identified (Gilmartin *et al.*, 1990). It will be of interest to analyze the promoter regions of the glycolate oxidase gene and their interactions with trans-acting factors. Comparison of the glycolate oxidase and rbcS gene systems may help us understand the mechanism of light-regulated gene expression in general as well as the expression patterns of the two genes observed in this study.

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References

Dean, C., Pichersky, E. and Dunsmuir, P. (1989) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **40**, 415.

Dedonder, A., Rethy, R., Fredericq, H., Montagu, M. V. and Krebbers, E. (1993) *Plant Physiol.* **101**, 801.

De Vries, S., Hoge, H. and Bisseling, T. (1988) in *Plant Molecular Biology Manual* (Gelvin, S. B. and Schiperoot, R. A. eds) pp. B6/1-5, Kluwer Academic Publishers, Dordrecht, The Netherlands.

Fluhr, R. and Chua, N.-H. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 2358.

Fritz, C. C., Herget, T., Wolter, F. P., Schell, J. and Schreier, P. H. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 4458.

Gerdes, H.-H. and Kindle, H. (1988) *Biochim. Biophys. Acta* **949**, 195.

Gilmartin, P. M., Sarokin, L., Memelink, J. and Chua, N.-H. (1990) *Plant Cell* **2**, 369.

Park, Y.-S., Choi, J. D. and Cho, N. J. (1992) *Kor. Biochem. J.* **25**, 219.

Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) Molecular Cloning, A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Silverthorne, J. and Tobin, E. M. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 1112.

Stiekema, W. J., Wimpee, C. F., Silverthorne, J. and Tobin, E. M. (1983) *Plant Physiol.* **72**, 717.

Volokita, M. and Somerville, C. R. (1987) *J. Biol. Chem.* **262**, 15825.

Wanner, L. A. and Gruissem, W. (1991) *Plant Cell* **3**, 1289.

Wehmeyer, B., Cashmore, A. R. and Schafer, E. (1990) *Plant Physiol.* **93**, 990.