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Wavelength Dependency of the Light-driven Transcriptional Activation of the Cucumber CPD Photolyase Gene

By

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K e y w o r d s : Action spectra, cyclobutane pyrimidine dimers, DNA photolyase (EC 4.1.99.3), ultraviolet light.

S u m m a r y

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UVB radiation (280 nm ~ 315 nm) is known to retard plant growth. DNA lesions are thought to be largely responsible for the growth inhibition due to UVB. Cyclobutane pyrimidine dimers (CPDs) constitute a major portion of UVB-induced DNA lesions. CPD-specific DNA photolyase (CPD photolyase) rapidly restores CPDs, rendering plants tolerant to UVB. We previously showed that the photolyase activity in cucumber leaves rises in the midst of the day when the solar UVB is intense, and that such diurnal fluctuation of the photolyase activity is attributable principally to light-dependent transcriptional activation of the CPD photolyase gene (*CsPHR*). In the present research, we examined the accumulation of the *CsPHR* transcripts under monochromatic light and showed that the *CsPHR* transcription is maximally induced by UVB with wavelengths around 310nm. It was surmised that the transcriptional activation is mediated by an unidentified UVB-specific photoreceptor.

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Introduction

The ultraviolet region of the solar radiation reaching the earth's surface is conventionally subdivided into UVA (320 nm ~ 400 nm) and UVB (290 nm ~ 320 nm). Disruption of the stratospheric ozone layer due to human activities would lead to a significant increase in the UVB intensity at the ground level (LARCHER 1975). UVB radiation is known to retard plant growth (TERAMURA 1983, TAKEUCHI & al. 1989). DNA lesions are thought to be largely responsible for the growth inhibition due to UVB (CALDWELL & al. 1986). Cyclobutane pyrimidine dimers (CPDs) constitute a major portion of UVB-induced DNA lesions (BRITT 1996).

CPDs are enzymatically restored via photoreactivation catalyzed by CPD photolyase. CPD photolyase, bound to its substrate, absorbs light in the UVA/blue region (photoreactivating light) and utilizes the light energy to achieve the restoration through subsequent transient electron transfer to the CPD (SANCER 1994). Many experimental data have indicated the physiological importance of CPD photolyase in plants. CPDs formed in plants are restored much more rapidly under photoreactivating light than in the dark (TAKEUCHI & al. 1996, TAYLOR & al. 1996). An *Arabidopsis* mutant lacking functional CPD photolyase, *uvr2-1*, is hypersensitive to UVB (LANDRY & al. 1997). These observations strongly support the idea that CPD photolyase is essential for the plant survival under UVB irradiation.

Many researches regarding the effect of UVB irradiation on plants have been conducted using cucumber (*Cucumis sativus* L.) plants (TAKEUCHI & al. 1989, NOUCHI 1993, KAWASHIMA & al. 2000). The first true leaf of a cucumber plant expands rapidly and horizontally, making itself suitable for light-irradiation experiments and observation of the effects of UVB irradiation on the leaf growth. We previously showed that the photolyase activity in cucumber leaves rises in the midst of the day when the solar UVB is intense, and that such diurnal elevation of the photolyase activity is attributable principally to light-dependent transcriptional activation of the CPD photolyase gene (*CsPHR*). The light-driven transcriptional activation of the photolyase gene was thought to be an adaptation strategy that plants have developed to tolerate the solar UVB (TAKAHASHI & al. 2002).

The purpose of the present study was to gain spectroscopic insights into the light-driven transcriptional activation of *CsPHR* by investigating its monochromatic action spectrum.

Material and Methods

Plant materials and growth

Prior to the polychromatic UV treatment, the seedlings of cucumber (*Cucumis sativus* L. cv. Hokushin) were first grown in a naturally lit glasshouse under controlled environment. The temperature and the relative humidity in the glasshouse were maintained at 25 ± 0.5 °C and 70 ± 5%, respectively. After the cotyledons had opened completely, the seedlings were transferred to an artificially lit growth cabinet on the 8th day after sowing. The growth cabinet was programmed for a temperature regime of 20 ± 0.5 °C during the day and 15 ± 0.5 °C at night, 12 h of light (6:00 – 18:00), and relative humidity at 70 ± 3%. Metal halide lamps (BOC Lamp, Mitsubishi Electric Corp., Tokyo, Japan) were used to provide the white light with wavelengths above 350 nm (Fig. 1a).

The photosynthetically active photon flux density (PPFD) at the plant height was 300 μmol m^{-2} s^{-1}, as measured with a quantum meter (LI-1000, Li-Cor, Lincoln, NE, USA). The seedlings were subjected to UV treatment on the 13th day after sowing.

For the monochromatic light irradiation, seedlings grown in the naturally lit glasshouse for 10 days were transferred to an artificially lit environment-controlled room, where the temperature and the relative humidity were maintained at 20 ± 0.5 °C and 60 ± 5%, respectively. White light was supplied with fluorescent lamps (FLR40S W/M, Toshiba Light Technology Corp., Tokyo, Japan) during the light period (6:00 – 18:00). The PPFD at the plant height was 200 μmol m^{-2} s^{-1}. The folded first true leaves opened and began to expand horizontally on the 11th day after sowing. On the 12th day, the plants were subjected to the monochromatic light irradiation.

The first true leaves were excised and frozen in liquid nitrogen immediately after the irradiation, then stored at –80 °C until the extraction of RNA or DNA.

Irradiation of polychromatic UV

Polychromatic UV (λ_{max} at 315 nm), in addition to white light, was irradiated using UV fluorescent lamps (FL20SE, Toshiba Light Technology Corp., Tokyo, Japan). Polyvinyl filters (Cutting Sheet 000C, Nakagawa Chemical, Co. Ltd., Tokyo, Japan) were used to remove light with wavelengths below 290 nm (Fig. 1). The UVB intensity measured with a UV monitor (MS210I, EKO Instruments Trading Co., Ltd., Tokyo, Japan) was 0.25 W m^{-2} at the plant height.

Irradiation of monochromatic light

Irradiation of monochromatic light was performed using the Okazaki Large Spectrograph (WATANABE & al. 1983). The plants with horizontally expanding first true leaves were exposed to vertically incident monochromatic light with wavelengths of 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 400, 450, and 500 nm for 4 hours, starting at 6:00. Monochromatic light of each wavelength was irradiated at 4 different intensities. The fluence rate was measured with a photon density meter (PFDM-200LX, Rayon Industrial Co. Ltd, Tokyo, Japan). Four plants were kept in the dark as controls in each experiment.

Quantification of CsPHR transcripts via quantitative RT-PCR

The *CsPHR* transcripts were quantified via quantitative RT-PCR according to TAKAHASHI & al. 2002. In brief, the total RNA (0.1 μg) extracted from the frozen leaf was subjected to *in vitro* reverse transcription using $pd(T)_{12\text{-}18}$ (Amersham Pharmacia Biotech, Inc., NJ, USA) as a primer. Then PCR was performed using specific primers for *CsPHR*. To evaluate the precision of RT-PCR, control reactions were performed with specific primers for 18S ribosomal RNA. The amount of amplified DNA fragments derived from the *CsPHR* transcripts was quantitated by performing Southern blot analysis using [α-^{32}P]dCTP-labeled *CsPHR* cDNA as a probe and measuring the radioactivity of the *CsPHR* hybridization signal.

Quantification of CPDs via ELISA

The amount of CPDs in the leaf was measured via an enzyme-linked immunosorbent assay (ELISA) using CPD-specific antibodies according to TAKAHASHI & al. 2002.

Analyses of action spectra

In order to obtain the action spectrum for the transcriptional activation of *CsPHR*, the transcript levels were normalized to the mean value for the 4 dark-control plants, then semilogarithmically regressed against the light intensity (Fig. 3a). The regression coefficient for each wavelength was calculated from the fluence-response plot. The experiment was repeated 6 times and the mean value of the regression coefficients was calculated for each wavelength. The mean values were normalized to the value at 310 nm and plotted against the wavelengths as induction efficiency (Fig. 3b).

In order to obtain the action spectrum for CPD accumulation in the leaf, the amount of CPDs, represented by A_{492}, was linearly regressed against the light intensity (Fig. 4a). The regres-

sion coefficient calculated for each wavelength was normalized to the value at 290 nm and plotted against the wavelength as CPD formation efficiency (Fig. 4b).

Results and Discussion

In our previous studies regarding the diurnal transcriptional fluctuation of *CsPHR*, the experiments were performed under white light with wavelengths longer than 350 nm (Fig. 1a). However, in the natural environment, plants receive sunlight containing UV with wavelengths longer than 290 nm. In the present study, we first investigated how the supplemental polychromatic UV ($\lambda > 290$ nm) irradiation (Fig. 1b) would affect the *CsPHR* transcription in the cucumber seedlings grown under white light. The supplemental UV did not chronologically

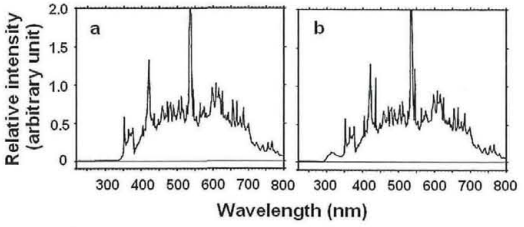

Fig. 1. Spectral irradiance for plant growth and UV treatment. **a**, Light spectrum for plant growth. **b**, Light spectrum for UV treatment.

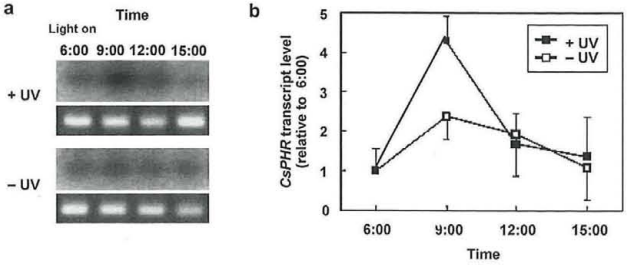

Fig. 2. Promotion of the *CsPHR* transcription by supplemental UV irradiation. **a,** Seedlings grown under white light (Fig. 1a) were subjected to supplementary UV (Fig. 1b) on the 13th day after sowing. *CsPHR* transcripts in the first true leaves were detected via RT-PCR followed by Southern hybridization (upper row). Validity of RT-PCR was confirmed by ethidium-bromide staining of the RT-PCR products derived from *18S rRNA* (bottom row). **b,** *CsPHR* transcript levels were quantitated by measuring the radioactivity. Solid and open squares represent values for UV-treated and control plants, respectively. Each bar indicates ±S.D. for at least 3 independent plants.

affect the diurnal pattern, the maximal transcript accumulation being observed at 9:00. On the other hand, the transcript level at 9:00 was enhanced approximately 2-

fold under the low-fluence UV (Fig. 2). It was, therefrom, surmised that plants are equipped with a UV-specific photosensory mechanism that leads to efficient expressional induction of the DNA-repair enzyme.

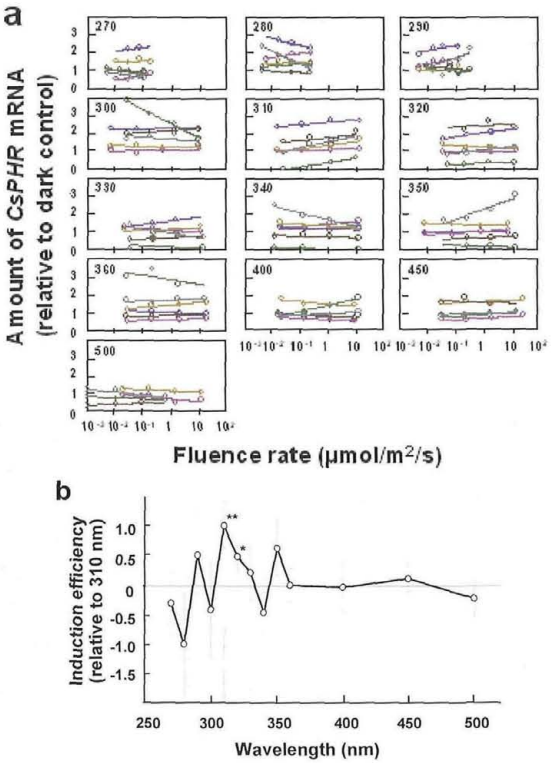

Fig. 3. Wavelength dependency of the *CsPHR* transcriptional activation. **a,** Fluence-response plots for accumulation of *CsPHR* transcripts. Wavelength of the incident monochromatic light is indicated at upper left corner. Experiments were repeated 6 times. Here different sets of experimental data are colored differently. In each experiment, *CsPHR* transcript levels in first leaves were normalized to the mean value for 4 dark-control plants and semilogarithmically regressed against the fluence rate. **b,** Action spectrum for the transcriptional activation of *CsPHR*. Regression coefficient for each wavelength was calculated from the fluence-response plots. The mean value for the regression coefficients obtained in repeated experiments was plotted against wavelength as induction efficiency. Each bar indicates ±S.E. Asterisks indicate significant differences at $P<0.1$ (*) and $P<0.05$ (**) determined by one-sample *t* test against the value zero.

We further investigated the wavelength specificity of the transcriptional activation in the UV / blue spectrum (270 - 500 nm) via irradiation of monochromatic light (Fig. 3a). The *CsPHR* transcription was maximally induced by UVB with wavelengths around 310nm. UVA (350 nm) seemed to slightly induce the

transcription, though no statistically significant difference determined by one-sample *t* test against the value zero at $P<0.1$ was found. No transcriptional activation was evident under blue light or under UV with wavelengths shorter than 300 nm.

What photoreceptors mediate the transcriptional activation by UVB? No photoreceptors have been identified for UVB though some experimental results implied their existence in plants. In the monochromatic action spectra previously obtained for phototropism (BASKIN & IINO 1987), stem growth inhibition (STEINMETZ & WELLMANN 1986, BALLARÉ & al. 1995), root growth inhibition (STEINMETZ & WELLMANN 1986), stomatal opening (EISINGER & al. 2000), and anthocyanin accumulation (YATSUHASHI & al. 1982), the peaks appeared in the UVC / short wavelength UVB spectrum (260 - 300 nm). These results gave rise to the hypothesis that DNA lesions trigger the UVB-specific signal transduction. Thus, in order to evaluate whether this hypothesis is compatible with the action spectra for the transcriptional activation of *CsPHR*, we examined the efficiency of CPD formation in the leaf under monochromatic UV irradiation (Fig. 4). The action spectrum for CPD formation critically differed from those for the transcriptional activation of *CsPHR*. For example, the most effective transcriptional activation was observed at 310 - 320 nm, where the CPD formation was negligible. Signal transduction arising from DNA lesions, therefore, does not

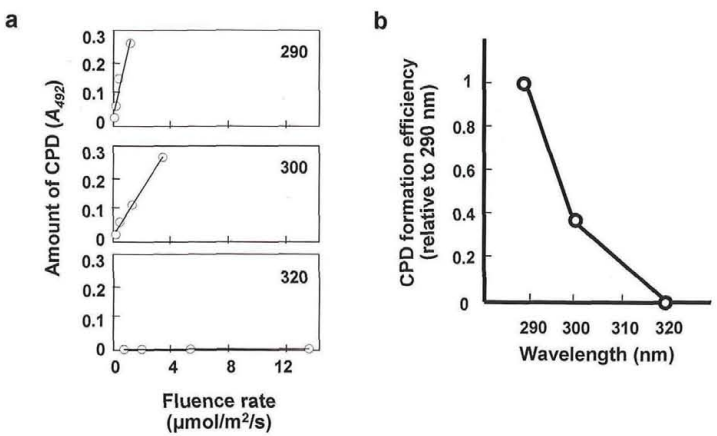

Fig. 4. Wavelength dependency of CPD formation in the leaf. **a,** Fluence-response plots for CPD accumulation under monochromatic UVB. Absorbance at 492 nm, which represented the amount of CPDs, was measured with Immuno Reader NJ-2201 (Nippon Intermed, Tokyo, Japan). Wavelengths (nm) are indicated at upper right corners. **b,** Action spectrum for CPD accumulation in the leaf. Regression coefficient was calculated for each wavelength and normalized to the value at 290 nm.

fully explain the promotion of the *CsPHR* transcription by UVB. The major induction of the photolyase expression, therefore, seems to be mediated by an unidenti-

fied photoreceptor specific for the long wavelength UVB around 310 nm in light-grown cucumber seedlings.

Even a small amount of short wavelength UVB (290 – 300 nm) causes DNA lesions in the foliar cells. Plants ingeniously express the DNA-repair enzyme in response to the less-damaging long wavelength UVB around 310 nm. The long wavelength UVB penetrates into the leaf in much larger amounts than the short wavelength UVB. In the natural environment, the long wavelength UVB contained in the sunlight reaches the ground prior to the short wavelength UVB. Induction of the photolyase expression by the long wavelength UVB, therefore, allows plants to adapt to sunlight by timely increasing the photoreactivation activity in the midst of the day. The unprecedented predominance of the UVB peak, given that the CPD photolyase is encoded by a single-copy gene (IOKI & al. 2003), makes the transcriptional activation event advantageous for further elucidation of UVB-specific photoperception mechanisms in plants and the signal transduction downstream of them.

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