Wavelength Effect on the Action of a $N$-Phenylimidoyl S-23142 and a Diphenylether Acifluorfen-Ethyl in Cotyledons of Cucumber (Cucumis sativus L.) Seedlings

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ABSTRACT

Specific wavelengths of light required for expression of phytotoxic activity of S-23142 (N-[4-chloro-2-fluoro-5-propargyloxy]phenyl-3,4,5,6-tetra-hydrophthalimide) and acifluorfen-ethyl (ethyl-5-[2-chloro-4-(tri-fluoromethyl)phenoxyl]-2-nitrobenzoic acid) were determined in cotyledons of cucumber seedlings using the Okazaki Large Spectrograph. Leakage of amino acids from the cotyledons was measured as an indication of the phytotoxic activity. The wavelength effects showed common major peaks of activity at 550 and 650 nanometers and a minor peak at 450 nanometers for both herbicides, indicating a common primary photoreaction. Concomitant application of DCMU (3,3′,4,4′-dichlorophenyl-1,1′-dimethylurea) with S-23142 had little influence on the effective wavelengths for S-23142 activity. Light of 450 and 650 nanometers was relatively less effective in achlorophyllous tissue grown in far red light than in green tissue. These results strongly suggest that the phototoxic action of S-23142 and diphenylethers involves multiple photoreactions and that one of the photoreceptor pigments may be chlorophyll or its related pigment, although photosynthesis is not involved.

S-23142 (N-[4-chloro-2-fluoro-5-propargyloxy]-phenyl-3,4,5,6-tetrahydrophthalimide) is an experimental herbicide which is highly active against a wide variety of weeds. Like p-nitro DPEs,3 represented by AFE, S-23142 requires light and oxygen for expression of its activity and induces peroxidation of PUFA which leads to damage of the membrane function (17). This mode of S-23142 action is similar to that of DPEs, although different photoreactions under the influence of the two types of herbicides could generate the identical phototoxic molecular species which causes common phenomena as the phytotoxicity of these herbicides.

The role of light in DPE action has also been unclear, as inconsistent experimental results have been reported on the involvement of photosynthesis and the effectiveness of red light in DPE action. Inhibition of photosynthesis had little or no effect on the activity of DPEs in cucumber (Cucumis sativus L.) cotyledons (3, 4, 14) and Chlamydomonas eugametos (6), but it partially reduced the activity in green bean (Phaseolus vulgaris L.) (15) and pea (Pisum sativum) (8) and completely nullified the activity in Scenedesmus actus (1, 10). Carotenoids have been implicated as photoreceptors for DPE action, because chlorophylls, yellow mutants (7, 11) or yellow plant materials obtained by chemical treatment (4) are sensitive to DPEs, and carotenoid-lacking, white materials are tolerant to them (2, 4, 7, 11, 12, 14). However, considerable evidence has accumulated that red light which cannot be absorbed by carotenoids is effective in DPE action (1, 5, 9, 16). There is also some discrepancy between two published wavelength effects on the activity of oxyfluorfen in wild buckwheat (19) and AFI in C. eugametos (5) in the red light region. Red light of wavelength longer than 615 nm did not induce the activity of oxyfluorfen but did induce that of AFI. The purpose of this study was to determine the specific wavelengths of light required for the activities of S-23142 and AFE, and to see whether any difference exists in the photoreactions involved in the action of these two different types of chemicals. The effect of DCMU on the wavelength effect was also examined to evaluate the involvement of photosynthesis.

MATERIALS AND METHODS

Plant Material. Cucumber seeds (Cucumis sativus L. cv Sagemi Hanpaku) were obtained from Takii Seed Co., Ltd. (Kyoto). Seedlings were grown on 5 ml of Hoagland solution solidified with 0.5% (w/v) agar in glass vials (27 mm diameter, 6 cm height) under a 14-h photoperiod for 4 d or under continuous FR for 3.5 d. The intensity of white fluorescent light and FR was 8 W/m² and 0.4 W/m², respectively. Each vial contained four seedlings. The temperature was 25°C throughout the experiments.

Herbicide Treatment. Herbicides were dissolved in acetone and kept as stock solutions. Herbicide solutions were prepared by adding the acetone stock solution to DDW. The final concentration of acetone was below 0.1% (v/v). Fifteen ml of herbicide solution was poured into each vial to submerge the seedlings and then was discarded after 1 h in darkness. The seedlings were incubated in darkness for an additional 3 h before 6 h of irradiation. This procedure enabled uniform wetting of the seedlings with herbicide solution and reduced errors in the replicates.

Light Treatment. The Okazaki Large Spectrograph provided monochromatic light irradiation with a 30 kW Xenon short arc lamp as the light source and threshold sample boxes (21). The threshold sample box is composed of two aluminum first face
mirrors and seven partially transmitting mirrors. It can thus project approximately parallel light beams having diameters of 80 nm vertically downward into seven separate compartments. Each compartment receives about 40% of the fluence rate of the preceding one in the range from 250 to 1000 nm. Detail of the instruments was described by Watanabe, et al. (21). Three replicated vials were irradiated simultaneously with monochromatic light of 250 to 850 nm, at 50-nm intervals and with three to four different photon fluence rates. The half-bandwidth of the irradiation was about 9 nm. Appropriate neutral density filters (Fujitok Corp. Tokyo, Japan) were placed on the openings of the sample boxes to adjust photon fluence rates of monochromatic light irradiation. The photon fluence rates of monochromatic light were measured with a photon density meter HK-1 (T Hashimoto, H Yatsuhashi, H Kato 1982 Abstract of Annual Meeting of JSPP, p 38), custom-made by the Institute of Physical and Chemical Research (Wako, Saitama, Japan). Twenty W white fluorescent lamps (Mitsubishi, FL 20 SW/NL) were used to provide white light and a wide range of red light, which was obtained with a 5-mm-thick red plastic filter (Acrylite 102, Mitsubishi Rayon Co. Ltd., Tokyo, Japan). FR was obtained from fluorescent FR lamps (Toshiba, FL 20S-FR-74) using a plastic film (IR-1, Koto Denki, Saitama, Japan) and a 5-mm-thick red plastic filter. The fluence rate of the white light and red light were measured with a UDT 181 radiometer equipped with a photodiode detector head and a radiometric filter (United Detector Technology Inc., Santa Monica, CA). All samples except the dark controls were irradiated for 6 h unless otherwise stated. After the irradiation, the seedlings were incubated for 14 h in the dark before determination of amino acid leakage. For handling in the dark, IR longer than 850 nm, which was obtained from a head lamp (National BF-172, Matsushita Electric) using a glass filter (IR-85, Hoya Corp., Tokyo, Japan) and a plastic film (Filmolux No. 87, Hans Neschen Co., Bückeburg, West Germany), and an IR viewer (Nocovision NVR 2015, NEC, Tokyo) were used.

Amino Acid Leakage. Six cotyledons, selected from four seedlings in each vial, were plunged into 6 ml of DDW and shaken gently for 2 h. A 0.5-ml portion of the bathing solution was sampled for amino acid determination. From the beginning of the herbicide treatment until the end of the sampling of the amino acid solution, all procedures were conducted in the dark. The amount of amino acid lost from the cotyledons was determined by a colorimetric reaction with ninhydrin according to the method of Yemm and Cocking (22). The difference in A570 between the irradiated sample and the dark control was expressed as the activity of the herbicides under the influence of light.

RESULTS

Effect of S-23142 and AFE on Amino Acid Leakage in Cucumber Cotyledons. Since solute leakage is one of the characteristic and quantitatively measurable parameters of phytotoxicity induced by DPE herbicides (13, 14, 18) and S-23142 (17), amino acid leakage from cucumber cotyledons was measured to determine the specific wavelengths required for their herbicidal activities. Cucumber seedlings grown in the dark for 4 d were treated with 3 μM S-23142 or 25 μM AFE and incubated for 4 h in the dark before irradiation. Preliminary experiments showed that this dark preincubation resulted in adequate incorporation of S-23142 into cotyledons, and that the effect of S-23142 on amino acid leakage was saturated at 3 μM (data not shown). The optimum effect of AFE, although lower than S-23142, was obtained at 25 μM (data not shown). The concentration of AFE could not be increased further because of its limited water solubility. Leakage of amino acids from the cotyledons treated with S-23142 increased with irradiation time and became saturated at 6 h irradiation, while 25 μM AFE required longer irradiation time for the saturation of its effect (Fig. 1). Therefore, seedlings were irradiated for 6 h to determine the wavelength effect on the activity of S-23142.

Wavelength Effect on the Action of S-23142 and AFE. The seedings treated with S-23142 or AFE were irradiated with various monochromatic lights of 250 to 850 nm for 6 h. The fluence-response relationship for the amino acid leakage from cucumber cotyledons in the presence of 3 μM S-23142 was obtained at each wavelength examined, some of the results are presented in Figure 2. The amino acid leakage induced by 3 μM S-23142 at the photon fluence rate of 3 × 1013 photon/cm2/s was determined and plotted for each wavelength. The wavelength effect showed major peaks at 550 and 650 nm and minor peaks at 450 and 384 nm (Fig. 3). Similarly, the wavelength effect on the action of AFE was determined at the photon fluence rate of 2 × 1014 photon/cm2/s between 350 to 750 nm, at 50-nm

![Fig. 1. Effect of S-23142 (3 μM) and AFE (25 μM) on leakage of amino acids from cotyledons of cucumber seedlings. Cucumber seedlings were treated with S-23142 or AFE in the dark and irradiated with white fluorescent light (6.6 W/m2) for various lengths of time. Amino acid leakage from cotyledons was determined, using the ninhydrin reaction, at 12 h after the end of the irradiation by measuring A570. Each point represents the average of three replications. Bars are SE.](image-url)
Fig. 3. Wavelength effect on leakage of amino acids from cotyledons of cucumber seedlings treated with 3 μM S-23142 and 25 μM AFE. Amino acid leakage at photon fluence rate of 3 × 10^{13} photon/cm^2-s for S-23142 and 2 × 10^{14} photon/cm^2-s for AFE was determined from the data with three replications as shown in Fig. 2. The interval of the wavelengths irradiated in an experiment was 50 nm and the results of several experiments were combined. Each open point represents the single determination. Each solid point is the average of two or three determinations.

Effect of DCMU on the Wavelength Effect. Since the wavelength effect on the action of S-23142 and AFE showed a major peak in red light, the effect of DCMU on the wavelength effect was examined to investigate a possible involvement of photosynthesis. Seedlings were treated with 130 μM DCMU plus 3 μM S-23142, then the wavelength effect on the amino acid leakage from cucumber cotyledons at the photon fluence rate of 3 × 10^{13} photon/cm^2-s was determined as described above. At the beginning of the irradiation, the cotyledons treated with 130 μM DCMU plus 3 μM S-23142 showed no photosynthetic oxygen evolution and the amino acid leakage from them was slightly lower than that from those treated with S-23142 alone (Table I). DCMU alone did not induce amino acid leakage under this experimental condition (Table I). Figure 4 shows the wavelength effect on the action of S-23142 in the presence of 130 μM DCMU. The action of S-23142 was slightly decreased by DCMU at all the wavelengths examined. However, the characteristics of the wavelength effect, in which major peaks were observed at 450, 550, and 650 nm, were essentially the same as those determined without DCMU. This result indicated that photosynthetic electron transport is not directly involved in the action of S-23142.

Wavelength Effect on S-23142 Action in FR-Grown Tissue. Since FR-grown tissue is highly sensitive to DPEs and has very little Chl (3), the wavelength effect on the action of S-23142 in FR-grown tissue was determined to find whether Chl could be the photoreceptor in the action of S-23142. Chl was absent from FR-grown tissue at the beginning of the irradiation (data not shown) and its formation proceeded during the 6 h of irradiation. S-23142 inhibited the formation of Chl, and the Chl content in cotyledons treated with 3 μM S-23142 at the end of 6-h irradiation period was less than 5% of that in untreated green tissue (Table II). Figure 4 shows the wavelength effect on the action of S-23142 at the photon fluence rate of 3 × 10^{13} photon/cm^2-s in FR-grown tissue. Although the amino acid leakage in FR-grown tissue should not be compared directly to that in green tissue as the size and thickness of them were different, peaks of S-23142 activity in FR-grown tissue were observed at 550 nm, as in green tissue, and at 400 nm. However, the peak at 450 nm observed in green tissue was lost, and the one at 650 nm was relatively lower than in green tissue. These findings strongly suggest that multiple photoreactions are involved in the action of S-23142 and that Chl or its related pigment is one of the photoreceptors.

**DISCUSSION**

Despite differences in their chemical structures, S-23142 and DPEs cause the same physiological and biochemical responses, such as lipid peroxidation, solute leakage, and stress ethylene production, in the presence of light and oxygen (17). Our present work clearly demonstrates that the photoreactions involved in the action of S-23142 and AFE are identical. Thus, S-23142 and AFE seem to have a common primary mechanism of action, which has not been fully clarified yet.

This study showed for the first time the precise wavelength effect on the DPE-type action in a higher plant. Ensmer and Hess (5) reported a wavelength effect on the action of AFM in the unicellular algae *Chlamydomonas eugametos*. AFM activity shows two major peaks at 450 and 660 nm and two minor peaks at 350 and 530 nm. Our present results (Fig. 3) also showed four corresponding peaks in the near UV, blue, green, and red light, although the major peaks were at 550 and 650 nm. As cucumber cotyledons are a rather thick type of tissue, it is likely that

Table 1. Effect of DCMU and S-23142 on Leakage of Amino Acid and Photosynthetic Oxygen Evolution in Cucumber Cotyledons

Cucumber seedlings were treated with herbicides as described in “Materials and Methods.” Cotyledon discs of 5-mm diameter were prepared at the beginning of the 6 h of irradiation with red light, and photosynthesis of the discs was measured with a Clark-type oxygen electrode. Leakage of amino acids from cotyledons was assayed as described in “Materials and Methods.” Each value is the average of three determinations.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>Photosynthetic Gross O₂ Evolution</th>
<th>Amino Acid Leakage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μM</td>
<td>μmol/dm²·h</td>
<td>A₅₇₀</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCMU</td>
<td>130</td>
<td>350*</td>
<td>0.153*</td>
</tr>
<tr>
<td>S-23142</td>
<td>3</td>
<td>316*</td>
<td>0.141*</td>
</tr>
<tr>
<td>S-23142 + DCMU</td>
<td>3 + 130</td>
<td>0*</td>
<td>1.123*</td>
</tr>
<tr>
<td></td>
<td>3 + 43</td>
<td>148ab</td>
<td>0.751e</td>
</tr>
<tr>
<td></td>
<td>3 + 14</td>
<td>315a</td>
<td>0.898ke</td>
</tr>
</tbody>
</table>

* Values within a column followed by the same letter are not significantly different at the 5% level according to Duncan's multiple range test.

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penetration of light into the cotyledon was greatly influenced by the masking effect of Chl and carotenoid in the surface layers of cells. Therefore, green light of 550 nm that is not effectively absorbed by Chl or carotenoid might be able to reach the cells inside the tissue more easily and more effectively induce the activity of S-23142 and AFE in cucumber cotyledons than blue and red light. Slight differences in the wavelengths of the peaks between these two systems do not seem to be significant because of the long irradiation time and the intervals of wavelengths examined.

One of the most important findings in the present work is that red light is effective in DPE-type action even in the presence of a sufficient amount of DCMU to completely inhibit photosynthesis of the tissue. Since studies showing the absence of an antagonistic effect of DCMU on DPE action had been conducted under white light (3, 6), there remained the possibility that photosynthesis was one of the multiple photoreactions which led to the expression of DPE action. However, our finding clearly eliminated this possibility and confirmed that photosynthesis is not involved in the action of S-23142. The slight decrease of S-23142 activity caused by DCMU in Figure 4 might be due to the lack of oxygen supply from PSII to the site of action. Our result also confirmed that a red light-absorbing pigment, other than carotenoids, works as a photoreceptor in the action of S-23142 and DPEs. Since red and blue lights are relatively less effective in FR-grown tissue than in green tissue, it is likely that Chl or its related pigment is the photoreceptor of red and blue light. No peaks suggesting the involvement of carotenoids in the action of S-23142 were found in Figure 4. Therefore, carotenoids do not seem to participate directly in the primary action of S-23142. However, the determination of the wavelength effect in another achlorophyllous tissue is needed to examine this further. More research is also needed to identify the receptor pigment of green light of 550 nm.

Wakabayashi et al. (20) postulated that the primary site of action of cyclic imide herbicides in higher plants would be inhibition of Chl formation and the role of light in the phytotoxic action would be related to the role of light in the induction of the Chl formation. If so, a similar wavelength effect on Chl formation to that on phytotoxic action must be obtained. Our preliminary determination of the wavelength effect on Chl formation in herbicide-untreated, FR-grown tissue showed peaks at 450 and 650 nm among the wavelengths of 350 to 700 nm examined at 50-nm intervals (data not shown). Our results do not support their hypothesis, because the corresponding peaks at 450 and 650 nm were not found in Figure 4, although S-23142 inhibited Chl formation in FR-grown tissue.

The fact that DPE is active in achlorophyllous plant tissue (3, 4, 7, 11) and our present results led us to conclude that the photoreceptor pigment in the action of S-23142 and DPEs is not a single pigment. Orr and Hess (14) proposed a scheme in which DPEs generate lipophylic free radicals that cause peroxidation of PUFA in membranes from the interaction of carotenoids and herbicide molecules. Our present results strongly suggest that S-23142 and DPEs are more likely to interact with Chl and related pigments than with carotenoids. Our working hypothesis, a modification of that of Orr and Hess (14), is that S-23142 causes formation of photosensitizer by directly affecting molecules of Chl or related pigments or by affecting their metabolic pathways.

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LITERATURE CITED

methyl, a diphenyl ether herbicide, in Chlamydomonas eugametos. Plant Physiol 77: 503–505