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*Short Communication*

# Ultraviolet Light-induced Formation of Pisatin and Phenylalanine Ammonia Lyase[1]

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The production of pisatin is induced[3] in pea tissue by various plant pathogens (4, 8). This *de novo* synthesis can also be induced by an array of chemicals (9, 19, 22), most of which are known to have an affinity for double stranded DNA. Also, most of the inducers of pisatin or phenylalanine ammonia lyase (PAL)[4], EC 4.3.1.5 (a key enzyme in the biosynthesis of pisatin) have the potential to change the conformation of DNA. The induction of both pisatin and PAL is dependent on new RNA and protein synthesis (9, 22).

We have proposed (9, 22) that the control of these cellular responses occurs at the gene transcription level and depends on the conformational state of the double stranded DNA (*i.e.*, the DNA becomes more transcribable either by dissociating a repressor component or by assuming a more desirable conformation for transcription).

If conformational changes in cellular DNA do, in fact, initiate this response, ultraviolet light which can dimerize thymidine bases and cause lesions in DNA (5, 23) should also influence this response. This paper describes the induced synthesis of pisatin and PAL by ultraviolet light.

## MATERIALS AND METHODS

**Irradiations.** A filtered mineral light lamp (Model No. R-51, Ultraviolet Products, Inc.) emitting primarily at 254 nm was used for the short wavelength ultraviolet source at a distance of 13 cm from the endocarp surface of split pea pods. A Black Ray lamp (Model B-100A, Ultraviolet Products, Inc.) emitting at 366 nm was used for the long wavelength source at a distance of 20 cm from the pods. A source of blue light (440–620 nm) was obtained by filtering a 120 v photo flood bulb through blue cellophane at a distance of 30 cm from the pods.

**Plant Material and Induction Treatments.** Immature Alaska pea (*Pisum sativum* L.) pods (less than 2 cm long) were harvested from a greenhouse (10:00 a.m.) while still enclosed in the blossom, to minimize microbial contamination. The pods (1 g/treatment) were split immediately and placed in sterile Petri dishes (60 mm diameter). After the uncovered Petri plate of pods was exposed to the designated irradiation, 1.5 ml of sterile deionized water was added. The covered plate then was placed in the dark for the 18- to 20-hr incubation period. Inhibitor solutions (0.2 ml), when applied, were mixed with the aqueous solution in the Petri plate to bring the final concentration of the inhibitors, cycloheximide and 6-methyl purine, to 0.01 mg/ml and 0.5 mg/ml, respectively.

**Phenylalanine Ammonia Lyase Extraction and Assay.** One gram of tissue was homogenized in a mortar with 3 ml of a 0.05 M borate buffer (containing 0.5% sodium ascorbate) at pH 8.8, 1 g glass beads, and 0.1 g Polyclar. This and subsequent extractive operations were carried out at 2 C. The homogenate was filtered through four layers of cheesecloth and centrifuged at 20,000*g* for 10 min. The supernatant was assayed immediately according to the procedure of Koukol and Conn (16), revised as follows: the reaction mixture contained 1.5 ml of enzyme homogenate, 20 $\mu$moles of L-phenylalanine ($2.8 \times 10^5$ dpm L-phenylalanine-U-$^{14}$C) and 200 $\mu$moles of borate buffer, pH 8.8, in a final volume of 2.7 ml. The mixture was incubated for 2 hr at 37 C.

**Extraction and Quantitation of Pisatin.** Pisatin was isolated as described previously (21). An ethanol extract of pod tissue was taken to dryness and further re-extracted with hexane or petroleum ether. The residue of the hexane extract was separated on silica gel thin layer plates. Pisatin was detected on a silica gel thin layer plate by converting it to anhydropisatin in HCl fumes. Anhydropisatin fluoresces under long wave ultraviolet light. Pisatin was quantitated on the basis of its absorbance at 309 nm.

## RESULTS AND DISCUSSION

The irradiation of excised pea pods with short wave ultraviolet light promotes subsequent increases in pisatin and PAL (Tables I and II). The pisatin-producing response can be triggered by relatively short exposures to ultraviolet light, and pisatin is detectable within 6 to 8 hr after irradiation. Increases in PAL activity can be measured 6 hr after irradiation. Maximal activity of PAL occurs 18 hr subsequent to irradiation.

As the time of ultraviolet irradiation is increased from 5 to 15 min, PAL activity increases from 3-fold to 6-fold. However, radiation in excess of 30 min becomes detrimental, decreasing the induction potential.

The action spectrum of light quality enhancing PAL and pisatin formation is limited. Long wave ultraviolet light (366 nm) does not stimulate either pisatin or PAL production.

The enhanced formation of PAL appears to be dependent upon new RNA and protein synthesis. The induction of PAL by ultraviolet light is readily inhibited when 6-methylpurine (0.5 mg/ml), a potent inhibitor of all RNA synthesis in

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[3] The term "inducer" in this paper will refer to an agent which can cause an increase in extractable PAL and pisatin.

[4] Abbreviation: PAL: phenylalanine ammonia lyase.

plants (3, 13), is applied within 1 hr after the cells were irradiated (Table III). 6-Methylpurine is less effective in blocking the induction process when the inhibitor is applied 3 or more hr after irradiation. This indicates that part of the message required for enhanced PAL formation is synthesized in the first few hours after irradiation.

Ultraviolet-induced increases in PAL can be inhibited with cycloheximide (0.01 mg/ml) when the inhibitor is applied within 3 hr after irradiation. This inhibitor of protein synthesis is unable to halt completely the induction process if applied 6 hr after irradiation, indicating that some of the enzyme is synthesized within 6 hr after irradiation.

It has been demonstrated that thymidine dimers occur in DNA irradiated by short wave ultraviolet light (26). Ultraviolet-induced alterations of cellular DNA can also be demonstrated *in vivo* (24). The role of ultraviolet-induced dimerization in mutation has been well established in *E. coli* (5). Dimerization readily changes the density of DNA (23) as well as the base ratio of newly synthesized RNA (17). Low doses of ultraviolet light also slightly stimulate protein synthesis (2). The photoreactivation (or repair) of the radiation-induced DNA damage occurs if cells are exposed to long wavelength ultraviolet light or short visible radiation, immediately following a dose of the short wave ultraviolet light (14).

Photoreactivation of ultraviolet-induced biological damage has also been reported in higher plants (25) (*e.g.*, ultraviolet-induced growth inhibition [15] and mutation [12] can be reduced or negated by immediate posttreatment with visible light). Long wave ultraviolet light (Table II) or blue light (Table IV) do not appear to negate the short wave induced formation of PAL in peas.

Ultraviolet-induced conformational changes in segments of the DNA (lesions resulting from thymidine dimers) may influence the state of repression of the genes in pea tissue. Such action is compatible with the hypothesis (22) presented previously to explain the gene-activating effect of DNA intercalating compounds. That is, changes in the conformation of certain DNA segments (presumably regulatory segments) may in some way increase DNA repressor dissociation or improve the template properties (*e.g.*, increase polymerase binding sites [10]) of certain genes.

Table I. *Effect of Long and Short Wave Ultraviolet Light on the Induction of Pisatin*

| Inducer | Pisatin[1] |
|---|---|
| | μg/g |
| Short wave (30 sec) | 52 |
| Short wave (1 min) | 46 |
| Short wave (5 min) | 63 |
| Short wave (10 min) | 94 |
| Short wave (15 min) | 52 |
| Short wave (30 min) | 66 |
| Long wave (30 sec) | n.d.[2] |
| Long wave (1 min) | n.d. |
| Long wave (5 min) | n.d. |
| Long wave (10 min) | n.d. |
| Long wave (15 min) | tr[2] |
| Long wave (30 min) | tr |
| $H_2O$ | n.d. |

[1] Pisatin was extracted from 1 g of pods 40 hr subsequent to irradiation. Pods were incubated in the dark at 22 C.

[2] n.d. = not detectable, tr = trace.

Table II. *Effect of Long and Short Wave Ultraviolet Light on the Induction of Phenylalanine Ammonia Lyase*

| Inducer | PAL Activity |
|---|---|
| | % of water-induced control[1] |
| None | 100 |
| Short wave (5 min) | 296 |
| Short wave (10 min) | 392 |
| Short wave (15 min) | 648 |
| Long wave (5 min) | 102 |
| Long wave (10 min) | 100 |
| Long wave (15 min) | 107 |
| Short wave (5 min)—long wave (15 min) | 302 |
| Short wave (5 min)—long wave (30 min) | 288 |

[1] Average PAL activity of $H_2O$ treated control is 0.284 μmoles cinnamic acid per g pea pod per hr at 37 C. After irradiation the pea tissue was incubated in the dark for 18 hr at 22 C.

Table III. *Effect of 6-Methylpurine and Cycloheximide on the Induction of PAL by Short Wave Ultraviolet Light (245 nm)*

| Inducer | Time of Inhibitory Application | PAL Activity |
|---|---|---|
| | | % of water-induced control[1] |
| $H_2O$ | | 100 |
| Ultraviolet (13 min) | | 739 |
| | 6 Methylpurine | |
| Ultraviolet (13 min) | plus 13 min | 155 |
| Ultraviolet (13 min) | plus 1 hr | 165 |
| Ultraviolet (13 min) | plus 3 hr | 246 |
| Ultraviolet (13 min) | plus 6 hr | 358 |
| Ultraviolet (13 min) | plus 10 hr | 583 |
| | Cycloheximide | |
| Ultraviolet (13 min) | plus 13 min | 123 |
| Ultraviolet (13 min) | plus 1 hr | 121 |
| Ultraviolet (13 min) | plus 3 hr | 137 |
| Ultraviolet (13 min) | plus 6 hr | 332 |
| Ultraviolet (13 min) | plus 10 hr | 484 |

[1] Average PAL activity of $H_2O$-treated control is 0.125 μmoles of cinnamic acid per g pea pod per hr at 37 C. After irradiation the pea tissue was incubated in the dark for 18 hr at 22 C.

Table IV. *Effect of Blue Light on Ultraviolet Light-induced Increases in PAL*

| Inducer | PAL Activity |
|---|---|
| | % of water-treated control[1] |
| $H_2O$ | 100 |
| Short wave ultraviolet (10 min) | 584 |
| Short wave ultraviolet (15 min) | 963 |
| Short wave ultraviolet (10 min)—blue (5 min) | 686 |
| Short wave ultraviolet (10 min)—blue (10 min) | 385 |
| Short wave ultraviolet (15 min)—blue (5 min) | 525 |
| Short wave ultraviolet (15 min)—blue (10 min) | 497 |
| Blue (5 min) | 93 |
| Blue (10 min) | 62 |
| Blue (5 min)—short wave ultraviolet (10 min) | 743 |
| Blue (5 min)—short wave ultraviolet (20 min) | 700 |

[1] Average PAL activity of $H_2O$-treated control is 0.029 μmoles of cinnamic acid per g pea pod per hr at 37 C. After irradiation the pea tissue was incubated in the dark for 18 hr at 22 C.

Short wave length ultraviolet light undoubtedly influences cellular components other than DNA. The enhanced formation of PAL and pisatin does not appear to result from gross cellular damage or wounding, since the external appearance of the pod and the ability of the cells in the pod tissue to elongate remains unaltered. Short wave ultraviolet light also enhances PAL formation in beans (Hadwiger, unpublished).

Increased PAL synthesis has also been induced in various plant tissues with visible light (1, 6, 7, 18, 20, 27). The hypotheses implicating the mediation of the phytochrome system are currently accepted (11). The induction of PAL activity with ultraviolet light apparently does not involve phytochrome. The relationship between the two systems remains to be assessed.

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