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THE EFFECT OF SHORT-TERM IRRADIATION ON KINETIN-INDUCED AMARANTHIN SYNTHESIS IN *AMARANTHUS TRICOLOR* SEEDLINGS*

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Abstract—The accumulation of amaranthin in kinetin-treated seedlings of *Amaranthus tricolor* is significantly increased following exposure to light. This enhancement, which appears to be phytochrome-controlled since it is saturated at low energy levels of irradiation and is far-red reversible, involves the synthesis of enzymes which mediate the effect of light on pigment formation. However, this does not depend on the direct intervention of photo-activated phytochrome at the DNA level.

INTRODUCTION

IT IS well known that light controls the biosynthesis of plant pigments, such as flavonoids[1] or carotenoids.[2] Also the synthesis of betacyanins is light-controlled and it has been shown that phytochrome is involved in amaranthin formation stimulated by short-term irradiation in *Amaranthus tricolor* seedlings.[3] More recently, it has been reported[4] that pigment synthesis is stimulated in darkness by kinetin (6-furfurylaminopurine) and this stimulation appears not to be related to the activation of phytochrome. The effects of both light and kinetin have been ascribed to gene activation, since amaranthin synthesis induced individually by the two factors is prevented by actinomycin D, an inhibitor of DNA-directed RNA synthesis, and also by inhibitors of protein synthesis (puromycin and chloramphenicol).[4–6]

In order to understand the regulatory mechanism of the synthesis of this pigment, we decided to investigate the effects of illumination on kinetin-treated seedlings, since in these conditions the genes involved in amaranthin synthesis are activated by the hormone without affecting the phytochrome status. Therefore, any effect of light thus observed must be ascribed to other causes than gene activation mediated by the photoactivated phytochrome. The results of these investigations are reported in the present paper.

RESULTS

In *Amaranthus tricolor* seedlings the effects on amaranthin synthesis of long-term irradiation treatments (8 hr or more) with white light are independent of phytochrome status, while those of short irradiations are far-red reversible and therefore mediated by the photoactivated phytochrome (Table 1).

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[3] M. PIATTELLI, M. GIUDICI DE NICOLA and V. CASTROGIOVANNI, *Phytochem.* **8**, 731 (1969).
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TABLE 1. EFFECT OF FAR-RED ON AMARANTHIN SYNTHESIS INDUCED BY LIGHT*

Treatment	Amaranthin/seedling (10^{-2} nmoles)†
3 hr white light	30·6 ± 0·7
3 hr white light plus 5 min far-red	21·7 ± 0·6
6 hr white light	54·4 ± 1·2
6 hr white light plus 5 min far-red	37·6 ± 0·8
8 hr white light	64·0 ± 1·4
8 hr white light plus 5 min far-red	63·9 + 1·9
24 hr white light	93·5 ± 1·9
24 hr white light plus 5 min far-red	93·4 ± 1·9

* 2-Day-old seedlings were irradiated with white light for different periods of time, treated with far-red at the end of the illumination and then returned to darkness. Pigment was determined 24 hr after the beginning of the light treatment.

† 90% confidence level.

Kinetin-treated seedlings irradiated for periods of time ranging from 15 min–6 hr synthesize more amaranthin than the dark-grown controls. The effect of 15 min light treatment on the time course of amaranthin accumulation in kinetin-treated seedlings is shown in Fig. 1.

The increase in pigment formation is nearly independent of the amount of light (Table 2) and is in part reversed by far-red (Table 3), indicating the involvement of phytochrome. However, seedlings exhibit no response to terminal inactivation of phytochrome when the

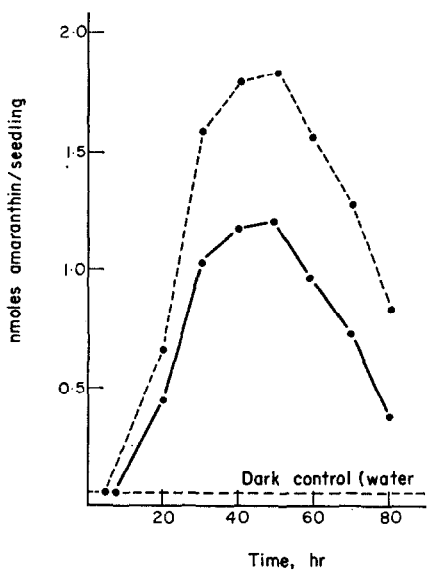

FIG. 1. TIME COURSE OF AMARANTHIN SYNTHESIS IN KINETIN-TREATED SEEDLINGS.
————, in darkness; - - - - - - -, 15 min white light at the onset of the experiment.

TABLE 2. EFFECT OF SHORT IRRADIATION TREATMENTS ON KINETIN-TREATED SEEDLINGS

Light treatment	Amaranthin/seedling (10^{-2} nmoles)*		
	Control seedlings (water)	Seedlings in kinetin†	Seedlings in kinetin‡
—	6·8 ± 0·1	68·8 ± 1·9	68·1 ± 1·8
15 min	12·1 ± 0·2	104·6 ± 3·2	108·4 ± 3·2
1 hr	21·4 ± 0·6	107·1 ± 3·3	112·3 ± 3·5
3 hr	30·6 ± 0·8	108·8 ± 3·3	113·3 ± 3·5
5 hr	42·9 ± 1·3	110·1 ± 3·5	113·6 ± 3·5
6 hr	54·4 ± 1·5	120·1 ± 3·6	113·5 ± 3·5

* 90% confidence level.

† 2-Day-old dark-grown seedlings were irradiated immediately after they had been transferred to kinetin solution (10 μg/ml).

‡ 2-Day-old dark-grown seedlings were irradiated 9 hr after they had been transferred to kinetin solution.

Pigment was determined 24 hr after the transfer to kinetin solution.

TABLE 3. EFFECT OF FAR-RED ON AMARANTHIN SYNTHESIS STIMULATED BY KINETIN AND A SHORT IRRADIATION TREATMENT*

Treatment	Amaranthin/seedling (10^{-2} nmoles)†
(a) Dark control (water)	6·8 ± 0·1
(b) 24 hr kinetin (darkness)	68·7 ± 1·9
(c) 24 hr kinetin + 15 min white light	104·6 ± 3·2
(d) 24 hr kinetin + 15 min white light followed by 5 min far-red	94·3 ± 2·5

*2-Day-old seedlings were treated with kinetin solution (10 μg/ml) for 24 hr (b). White light was given at the start of the kinetin application (c); this light treatment was immediately followed by 5 min of far-red (d). Pigment was determined at the end of the treatment.

† 90% confidence level.

TABLE 4. TIME DEPENDENCE OF REVERSIBILITY BY FAR-RED OF THE EFFECT OF A SHORT IRRADIATION TREATMENT ON KINETIN-INDUCED AMARANTHIN SYNTHESIS*

Treatment	Amaranthin/seedling (10^{-2} nmoles)†
(a) 24 hr kinetin	68·7 ± 1·9
(b) 24 hr kinetin plus 15 min white light	104·6 ± 3·2
(c) 24 hr kinetin plus 15 min white light plus 5 min far-red (0)	82·1 ± 2·5
(d) 24 hr kinetin plus 15 min white light plus 5 min far-red (+ 3)	82·2 ± 2·5
(e) 24 hr kinetin plus 15 min white light plus 5 min far-red (+ 7)	82·1 ± 2·5
(f) 24 hr kinetin plus 15 min white light plus 5 min far-red (+ 9)	104·6 ± 3·2

* 2-Day-old seedlings were transferred to kinetin solution (10 μg/ml) and irradiated with white light for 15 min in (b). In experiments (c) to (f) far-red (5 min) was given immediately (0), 3 hr (+ 3), 7 hr (+ 7) or 9 hr (+ 9) after the end of the white-light treatment. Pigment was determined 24 hr after the onset of the kinetin application.

† 90% confidence level.

far-red treatment is delayed by 9 hr or more from the end of the illumination (Table 4). Formation of the pigment is also enhanced if the irradiation is given before the moment of application of the hormone, provided that the length of the period of time between the two treatments does not exceed 8 hr (Table 5).

Seedlings irradiated 9 hr after kinetin application, i.e. when the hormone has completed its activating effect on the genes,[4] synthesize more amaranthin than dark-grown controls and this increase over the control is totally blocked by puromycin (Table 6) but not affected by actinomycin D (Table 7).

DISCUSSION

Actinomycin D inhibits the phytochrome-mediated synthesis of anthocyanins in *Sinapis alba* seedlings[7] and therefore it has been suggested that the photoactivated pigment acts at the gene level. The light-induced amaranthin synthesis in *A. tricolor* seedlings, which is blocked by actinomycin D, has been similarly considered to be mediated by phytochrome through gene activation.[5] However, it has not been conclusively proved that DNA is the primary locus of phytochrome action.

The results described in this paper show that light enhances amaranthin synthesis in kinetin-treated seedlings, in which the genic system responsible for the amaranthin synthesis has been already activated. This enhancement is far-red reversible and saturated at low energies of irradiation, and therefore appears to be phytochrome-controlled. However, it cannot be ascribed to gene activation since this has been already attained by the preliminary treatment with the hormone. Moreover, the fact that this enhancement is completely blocked by puromycin, a specific inhibitor of translation (*m*RNA-dependent protein synthesis), while it is unaffected by actinomycin D, an inhibitor of transcription (DNA-directed RNA synthesis) is evidence that the action of P_{730} involves the synthesis of the enzymes mediating the effect of light on amaranthin formation but this does not depend on a direct intervention of phytochrome at DNA level.

These results point to the possibility that when genes are not previously activated, i.e. in the absence of the hormone, light acts at two different levels, transcription and translation, in two different modes. In fact, while the effect on translation appears to be due to P_{730}, that on transcription seems to be mediated by other photoreceptor(s) than phytochrome, since complete gene activation by light requires much longer irradiation (approx. 6 hr, as estimated from the fact that after this time actinomycin D is no longer effective in blocking the light activation)[8] than that required for the activation of phytochrome (15 min). Accordingly, while in seedlings whose gene system has been previously activated by kinetin the increase in pigment formation is independent of the duration of the irradiation, a dependence of amaranthin accumulation on the amount of light is observed in control seedlings.

Since the effect of a brief irradiation on kinetin-treated seedlings remains far-red reversible for approximately 8 hr, it can be deduced that in the plant material under investigation the life-time of active phytochrome does not exceed this value. This is confirmed by the fact that when light is given before the application of kinetin, an enhancing effect is observed provided that the time interval between the application of the two factors is no longer than 8 hr. It is interesting in this connexion to note that Leff[9] found that short-term irradiation

[7] H. MOHR, *Photochem. Photobiol.* **5**, 469 (1966).
[8] M. GIUDICI DE NICOLA and M. PIATTELLI (unpublished results).
[9] J. LEFF, *Plant Physiol.* **39**, 299 (1964).

TABLE 5. EFFECT OF TIME APPLICATION OF A SHORT IRRADIATION TREATMENT ON KINETIN-TREATED SEEDLINGS*

Treatment	Amaranthin/seedling (10^{-2} nmoles)†
(a) Kinetin	64·6 ± 1·9
(b) Kinetin plus 15 min white light (0)	104·7 ± 3·2
(c) Kinetin plus 15 min white light (− 3)	103·1 ± 3·2
(d) Kinetin plus 15 min white light (− 7)	104·1 ± 3·2
(e) Kinetin plus 15 min white light (− 9)	64·7 ± 1·9

* 2-Day-old seedlings were treated with kinetin solution (10 μg/ml) for 24 hr. Light treatment was given at the start of kinetin application in (b); in experiments (c) to (e) seedlings were irradiated 3 hr (− 3), 7 hr (− 7) or 9 hr (− 9) before the transfer to kinetin solution. Pigment was determined at the end of the treatment.

† 90% confidence level.

TABLE 6. EFFECT OF PUROMYCIN ON AMARANTHIN SYNTHESIS STIMULATED BY KINETIN AND A SHORT IRRADIATION TREATMENT*

Treatment	Amaranthin/seedling (10^{-2} nmoles)†
(a) Dark control (water)	6·8 ± 0·1
(b) 24 hr kinetin (10 μg/ml)	68·7 ± 1·9
(c) 24 hr kinetin (10 μg/ml) plus puromycin (100 μg/ml)	10·7 ± 0·3
(d) 9 hr kinetin-15 hr kinetin plus puromycin	68·0 ± 1·9
(e) 24 hr kinetin plus 15 min white light (+ 9 hr)	104·6 ± 3·0
(f) 9 hr kinetin plus 15 min white light (+ 9 hr) − 15 hr puromycin plus kinetin	67·9 ± 2·0

* Seedlings were (b) incubated in kinetin for 24 hr, (c) incubated in a solution of kinetin and puromycin, (d) incubated for 9 hr in kinetin and then transferred to a solution containing both the hormone and the antibiotic, (e) incubated in kinetin solution and irradiated (15 min) 9 hr after the application of the hormone, or (f) incubated for 9 hr in kinetin, irradiated for 15 min and then transferred to the solution containing both the hormone and the antibiotic. Pigment was determined at the end of the treatment.

† 90% confidence level.

TABLE 7. EFFECT OF ACTINOMYCIN D ON AMARANTHIN SYNTHESIS STIMULATED BY KINETIN AND A SHORT IRRADIATION TREATMENT*

Treatment	Amaranthin/seedling (10^{-2} nmoles)†
(a) Dark control (water)	6·8 ± 0·1
(b) 24 hr kinetin	68·7 ± 1·9
(c) 24 hr kinetin plus actinomycin D	27·5 ± 1·4
(d) 9 hr kinetin-15 hr kinetin plus actinomycin D	67·7 ± 2·0
(e) 24 hr kinetin plus 15 min white light (+ 9 hr)	108·8 ± 3·5
(f) 9 hr kinetin plus 15 min white light (+ 9 hr) − 15 hr actinomycin plus kinetin	107·1 ± 4·0

* Seedlings were (b) incubated in kinetin (10 μg/ml) for 24 hr, (c) incubated in a solution of kinetin and actinomycin (both at concentration of 10 μg/ml), (d) incubated for 9 hr in kinetin and then transferred to the solution containing both the hormone and the antibiotic, (e) incubated in kinetin and irradiated (15 min) 9 hr after the application of the hormone, or (f) incubated for 9 hr in kinetin, irradiated for 15 min and then transferred to the solution containing both the hormone and the antibiotic. Pigment was determined at the end of the treatment.

† 90% confidence level.

strongly increases the percentage of germination of kinetin-treated lettuce seeds and the effect was observed also when the light treatment was given before the hormone application, provided that the time interval did not exceed 8 hr. These data can be rationalized by assuming that this light effect is mediated by photoactivated phytochrome, whose life-time is also 8 hr in this case.

Our results point to the conclusion that photoactivated phytochrome acts on amaranthin formation uniquely through the stimulation of protein synthesis, which can take place only when the gene activation needed for the production of the relevant mRNAs has been attained, by means of administration of kinetin or by a light treatment longer than that just sufficient for the activation of phytochrome. In this respect it is worth remarking that the stimulation by low levels of light of amino acid incorporation by ribosomes from dark-grown plants reported by Williams and Novelli[10] shows the typical features of a phytochrome-mediated response and therefore the effect of this photoreceptor on protein synthesis described in the present paper is perhaps not restricted to the material we investigated.

EXPERIMENTAL

Plant material. Seeds of *Amaranthus tricolor* were placed on two sheets of Whatman No. 1 filter paper moistened with tap water (14 ml) in 10-cm Petri dishes in darkness at 28°. A cool-white fluorescent source giving 5000 lx at seedling level was used. The far-red source was described previously.[3]

Administration of kinetin and antibiotics, and amaranthin estimation. Administration of kinetin and antibiotics[4] and the determination of amaranthin[3] was carried out as described previously. Ten replicates were used in all experiments and each experiment was repeated at least six times.

[10] O. R. WILLIAMS and D. NOVELLI, *Biochim. Biophys. Acta* **135,** 183 (1968).

Key Word Index—Amaranthus tricolor; Amaranthaceae; betalains; amaranthin synthesis; kinetin; light effects.