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# THE EFFECT OF KINETIN ON AMARANTHIN SYNTHESIS IN *AMARANTHUS TRICOLOR* IN DARKNESS*

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**Abstract**—Amaranthin synthesis in *Amaranthus tricolor* seedlings is stimulated by kinetin in darkness. This stimulation, which does not depend on the phytochrome status, is inhibited by actinomycin D and puromycin. The kinetin-induced amaranthin synthesis can be interpreted on the basis of gene activation.

## INTRODUCTION

KINETIN (6-furfurylaminopurine) has been found[1] to induce betacyanin formation in seedlings of *Amaranthus retroflexus* in the dark. Köhler[2] has reported that the light-stimulated synthesis of amaranthin in *A. caudatus* var. *viridis* was markedly increased by simultaneous administration of kinetin and tyrosine. However, no hypothesis as to the mechanism of kinetin action has been put forward. More recently, it has been established[3, 4] that the light-stimulated amaranthin synthesis in *A. tricolor* seedlings is controlled by phytochrome, probably through an activation of potentially active genes.

The purpose of the present investigation was to determine whether pigment formation in this plant material would be induced by kinetin in the dark and whether this effect could be interpreted on the basis of gene activation.

## RESULTS AND DISCUSSION

When seedlings of *A. tricolor* are incubated in kinetin solution in darkness a stimulation of pigment synthesis depending on the concentration of the hormone is observed (Fig. 1).

A 20 $\mu$g/ml concentration was found the most effective in enhancing pigment synthesis, but it caused an irregular development of the seedlings. Therefore, in all experiments a concentration of 10 $\mu$g/ml was used, which apparently does not damage the seedlings.

The effect of kinetin on pigment formation is also influenced by the age of the seedling and the largest amount of amaranthin is produced when kinetin is applied to 1-day-old seedlings (Table 1). However, to overcome difficulties in transplantation, 2-day-old seedlings were used in all experiments.

The amaranthin synthesis shows a lag phase of about 10 hr from the application of kinetin and attains a maximum at 48 hr. After this time a constant decrease of amaranthin was observed (Fig. 2).

As previously reported,[3] amaranthin formation in *A. tricolor* can be induced by light through the activation of phytochrome. In order to ascertain if the action of kinetin is also

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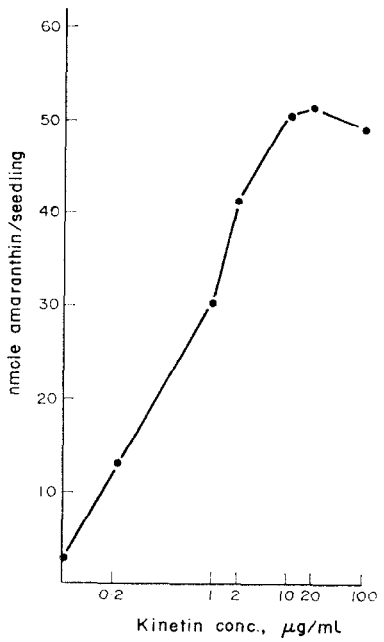


FIG. 1. CONCENTRATION DEPENDENCE OF KINETIN EFFECT ON AMARANTHIN SYNTHESIS.

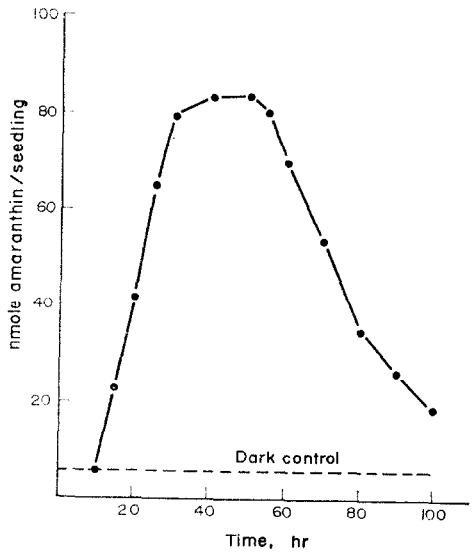


FIG. 2. TIME-COURSE OF KINETIN-INDUCED AMARANTHIN SYNTHESIS.

TABLE 1. EFFECT OF THE AGE OF *Amaranthus tricolor* SEEDLINGS ON THE KINETIN-INDUCED AMARANTHIN SYNTHESIS

| Kinetin (10 μg/ml) applied | Amaranthin/seedling (nmoles) |
|---|---|
| (a) at sowing | 62·90 ± 5·9 |
| (b) 24 hr after sowing | 102·32 ± 9·5 |
| (c) 48 hr after sowing | 63·72 ± 6·6 |

In experiments (a) and (b) the amount of amaranthin was determined 72 hr after sowing. In experiment (c) pigment was determined 96 hr after sowing.

mediated by phytochrome, seedlings were treated with the hormone and irradiated with far-red which produces the conversion $P_{730} \rightarrow P_{680}$. Since in all the conditions employed far-red irradiation did not modify the kinetin-induced amaranthin synthesis (Table 2), this appears not to be related to the activation of phytochrome, in that differing from the light-induced synthesis.

TABLE 2. EFFECT OF FAR-RED LIGHT ON AMARANTHIN PRODUCTION INDUCED BY KINETIN

| Treatment | Amaranthin/seedling (nmoles) |
|---|---|
| (a) Control (water) | 3·0 ± 0·2 |
| (b) Control (water plus 5 min far-red) | 3·0 ± 0·2 |
| (c) 24 hr Kinetin | 53·72 ± 3·1 |
| (d) 24 hr Kinetin plus 5 min far-red | 52·28 ± 2·9 |
| (e) 3 hr Kinetin–21 hr water | 25·23 ± 1·3 |
| (f) 3 hr Kinetin–21 hr water plus 5 min far-red (0) | 25·06 ± 1·2 |
| (g) 3 hr Kinetin–21 hr water plus 5 min far-red (+3) | 26·05 ± 1·4 |
| (h) 3 hr Kinetin–21 hr water plus 5 min far-red (+6) | 25·48 ± 1·3 |
| (i) 24 hr Kinetin plus 3 far-red treatments (0, +1, + 2 hr; 5 min each) | 52·80 ± 3·0 |
| (l) 3 hr Kinetin–21 hr water plus 3 far-red treatments (0, +1, + 2 hr; 5 min each) | 25·70 ± 1·5 |

2-Day-old seedlings were treated with kinetin solution (10 μg/ml) for 24 hr (c and d) or incubated in kinetin for 3 hr and then transferred to tap water (e–h). Far-red light was given at the start of kinetin application in d; in experiments f–h seedlings were irradiated immediately (0), 3 hr (+3) or 6 hr (+6) after they had been transferred to water. In experiments (i) and (l) seedlings were irradiated with far-red three times at 1-hr intervals. Pigment was determined at the end of the treatment.

Experiments to see if the effect of kinetin is at molecular level were carried out with actinomycin D, which inhibits DNA-dependent synthesis of RNA by combining with the DNA, and puromycin, an inhibitor of protein synthesis which prevents the formation of the polypeptide on ribosomes. The kinetin-induced amaranthin synthesis was completely suppressed when actinomycin D was applied before kinetin. When the antibiotic was given at increasing times after the onset of kinetin application, its effectiveness was progressively smaller and amaranthin synthesis was not blocked at all when actinomycin D was applied at the end of the lag phase (Table 3).

This suggests that the 'message' for the amaranthin synthesizing system may have been induced shortly after the kinetin application and that once it is produced, a recurring continuous assembly of this system is not necessary.

TABLE 3. INHIBITION BY ACTINOMYCIN D OF KINETIN-INDUCED AMARANTHIN SYNTHESIS AS FUNCTION OF THE TIME OF APPLICATION OF THE ANTIBIOTIC

| Treatment | Amaranthin/seedling (nmoles) |
|---|---|
| (a) 3 hr actinomycin D–24 hr actinomycin D plus kinetin | 3·0 ± 0·2 |
| (b) 24 hr actinomycin D plus kinetin | 19·87 ± 1·2 |
| (c) 3 hr Kinetin–21 hr actinomycin D plus kinetin | 35·45 ± 1·8 |
| (d) 5 hr Kinetin–19 hr actinomycin D plus kinetin | 40·82 ± 2·4 |
| (e) 7 hr Kinetin–17 hr actinomycin D plus kinetin | 46·19 ± 2·7 |
| (f) 9 hr Kinetin–15 hr actinomycin D plus kinetin | 53·72 ± 3·1 |
| (g) 24 hr Kinetin | 53·72 ± 3·1 |
| (h) Control (water) | 3·0 ± 0·2 |

Seedlings were (a) incubated for 3 hr in actinomycin D (10 μg/ml) and then transferred to a solution of actinomycin D and kinetin (both at concentration of 10 μg/ml), (b) incubated in the solution containing both the hormone and the antibiotic, or (c–f) incubated in kinetin solution for various times (3, 5, 7 and 9 hr) and then transferred to the solution of kinetin and actinomycin. Seedlings (g) were incubated 24 hr in kinetin. Amaranthin was determined at the end of the treatment. The data are averages from three different experiments.

Since puromycin applied simultaneously with kinetin almost completely blocks the formation of amaranthin (Table 4), it can be also inferred that the hormone induces *de novo* synthesis of proteins related to the pigment production.

TABLE 4. EFFECT OF PUROMYCIN ON AMARANTHIN PRODUCTION INDUCED BY KINETIN

| Treatment | Amaranthin/seedling (nmoles) |
|---|---|
| Kinetin (10 μg/ml) | 53·72 ± 3·1 |
| Kinetin (10 μg/ml) plus puromycin (100 μg/ml) | 10·70 ± 0·5 |
| Control (water) | 3·0 ± 0·2 |
| Control (puromycin) | 3·0 ± 0·2 |

2-Day-old seedlings were incubated for 24 hr in the various solutions after which time the pigment was determined.

From the results reported in the present paper it appears that amaranthin formation is induced by kinetin through a stimulation of RNA and protein synthesis. Such a hypothesis is in agreement with many reports on the effect of kinetin in plant tissues.[5–8] Therefore, kinetin-induced amaranthin synthesis can be ascribed to gene activation, similarly to the light-induced synthesis of the pigment. However phytochrome, which mediates the light-stimulated synthesis, is not implicated in the kinetin-induced synthesis.

## EXPERIMENTAL

### *Plant Material*

Seeds of *Amaranthus tricolor* were germinated on two layers of water-saturated filter paper in 10-cm Petri dishes and held in complete darkness at constant temperature (28°). All the treatments were carried out at the same temperature.

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*Light Source*

Far-red source was as previously described.[3]

*Administration of Kinetin*

Treatment with kinetin was carried out by submerging the seedlings in the hormone solution (10 $\mu$g/ml). When the treatment lasted longer than 12 hr, at the end of this period the seedlings were transferred to Petri dishes on filter paper moistened with the same solution and kept in darkness.

*Administration of Antibiotics*

In experiments with actinomycin D, dark-grown 2-day-old seedlings from which the radicle had been removed were used since, as previously reported,[4] the antibiotic is not absorbed by the roots.

Experiments with puromycin were carried out on intact seedlings. No significant difference was observed by using rootless seedlings.

*Determination of Amaranthin*

The quantitative determination of amaranthin was carried out as previously reported.[3]