Phytochemistry, 1970, Vol. 9, pp. 785 to 789. Pergamon Press. Printed in England.

THE INHIBITION BY ACTINOMYCIN D AND PUROMYCIN OF LIGHT-STIMULATED AMARANTHIN SYNTHESIS*

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(*Received* 17 *July* 1969)

Abstract—Actinomycin D and puromycin inhibit the light-stimulated synthesis of amaranthin in *Amaranthus tricolor* seedlings. This result suggests that the phytochrome-mediated formation of the pigment is controlled by P_{730} through the activation of potentially active genes, in agreement with Mohr's hypothesis on the mode of action of phytochrome.

INTRODUCTION

PHYTOCHROME is considered as the photoreceptor for red and far-red reversible reactions in plants and P_{730} appears to be its activated form. According to Mohr *et al.*[1-5] "positive" photoresponses, characterized by a promotion of biosynthetic processes (e.g. anthocyanin synthesis), can be explained by an activation or derepression of potentially active genes caused by P_{730}. This hypothesis has been tested with respect to particular "positive" photoresponses such as enlargement of mustard (*Sinapis alba*) cotyledons and synthesis of anthocyanins in mustard seedlings.

Recently,[6] amaranthin formation in *Amaranthus tricolor* seedlings has been shown to be subject to red/far-red reversible photocontrol. In the present paper results obtained in the study of the inhibition of amaranthin biosynthesis in *A. tricolor* by actinomycin D and puromycin are discussed on the basis of Mohr's hypothesis of differential gene activation.

RESULTS AND DISCUSSION

In darkness, *Amaranthus tricolor* synthesizes a very small amount of amaranthin ($2{\cdot}5 \times 10^{-9}$ moles/seedling). Under fluorescent light, after a lag period of about 4 hr, a large quantity of the pigment is produced. Actinomycin, a strong inhibitor of the DNA-dependent RNA polymerase reaction, applied at 10 μg/ml during the lag period (3 hr after the onset of light), reduced the rate of the light-induced amaranthin synthesis, which was further depressed when the antibiotic was given at the onset of irradiation (Fig. 1). Seedlings were not apparently damaged by the antibiotic treatment. However, in contrast to anthocyanin synthesis in mustard, some amaranthin is always formed, even with higher concentrations of actinomycin

* This work was supported by the Consiglio Nazionale delle Ricerche.

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D (to 200 μg/ml) or longer incubation periods (3 hr). The residual synthesis, equal to approximately 30 per cent of the control value, may correspond to actinomycin-resistant RNA synthesis.

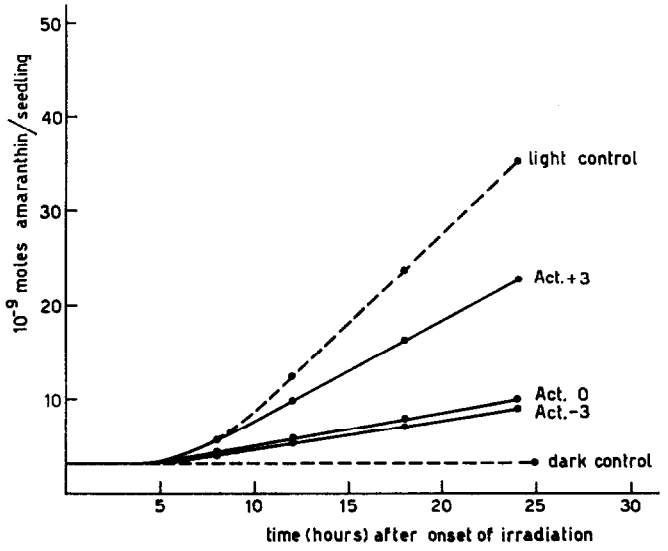

FIG. 1. INHIBITION OF THE LIGHT-INDUCED AMARANTHIN SYNTHESIS BY ACTINOMYCIN D (10 μg/ml). The antibiotic was applied 3 hr after the onset of irradiation (act. + 3), at the onset of irradiation (act. 0) and 3 hr before the onset of irradiation (act. −3).

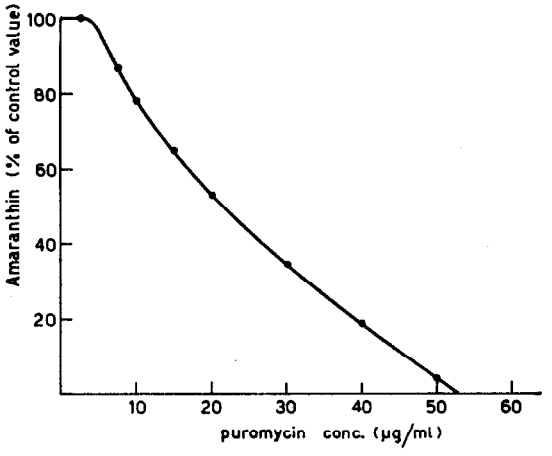

FIG. 2. CONCENTRATION DEPENDENCE OF PUROMYCIN EFFECT ON THE AMARANTHIN SYNTHESIS. Seedlings were irradiated 24 hr and the antibiotic applied at the onset of illumination.

Amaranthin formation was also depressed by the application of puromycin, a highly specific inhibitor of protein synthesis behaving as a competitive substrate for the peptidyl transferase. The intensity of the inhibitory effect of the antibiotic applied at the onset of light depends on its concentration and a total inhibition was observed with a concentration of 50 μg/ml (Fig. 2). The inhibiting effect of puromycin is also dependent on the time of its

addition to the seedlings and is greatly reduced when the antibiotic is applied after the onset of irradiation (Table 1). If the hypothesis that P_{730} acts through differential gene activation is correct, this can be interpreted assuming that the enzymes involved in amaranthin synthesis, which are formed under the influence of phytochrome, have a relatively long "life-time".

In order to estimate the "life-time" of the mRNA which controls the synthesis of these enzymes, seedlings of *A. tricolor* after 12 hr of illumination with fluorescent light were irradiated with far-red (5 min) to establish the $P_{730} \rightleftarrows P_{660}$ equilibrium at 730 nm and then darkened. Since the low concentration of P_{730} thus attained at the moment of darkening will be rapidly

TABLE 1. EFFECT OF PUROMYCIN ON THE AMARANTHIN SYNTHESIS IN CONTINUOUS LIGHT

Treatment	Amaranthin/seedling (moles × 10^{-9})
Dark control	3·2
Light control (24-hr light)	41·8
24-hr light + puromycin (0 hr)	3·6
24-hr light + puromycin (+2 hr)	18·1
24-hr light + puromycin (+4 hr)	28·0

The antibiotic (50 μg/ml) was applied at the onset of illumination (0 hr), 2 hr (+2 hr) and 4 hr (+4 hr) after the onset of light.

TABLE 2. EFFECT OF PUROMYCIN (50 μg/ml) ON THE DARK ACCUMULATION OF AMARANTHIN

Treatment	Amaranthin/seedling (moles × 10^{-9})
Dark control	3·0
Light control (12-hr fluorescent light followed by 24 hr of darkness)*	31·0
Light treatment as above; puromycin added at the moment of darkening	24·2
Light treatment as above; puromycin added at the 10th hour of the dark period	26·1
Light treatment as above; puromycin added at the 14th hour of the dark period	28·0

* At the end of the light period seedlings were irradiated with far-red (5 min).

destroyed, the increase of the amaranthin content of the seedlings which was observed during the subsequent 24 hr must be due to the enzymes already present at the moment of darkening and, if the mRNA is stable, in part also to the additional amounts of enzymes synthesized during the dark period. Addition of puromycin at the moment of darkening significantly depressed the amaranthin accumulation, thus showing that the mRNA has a relatively long "life-time" (Fig. 3).

Confirmatory evidence was obtained as follows; seedlings were irradiated 12 hr with fluorescent light, briefly (5 min) exposed to far-red and then darkened. Puromycin was added 10 or 14 hr after the moment of darkening and the amaranthin content was determined at the completion of the dark period (24 hr). The inhibition which was observed in both cases shows

that the enzymes for pigment synthesis are still being produced a relatively long time after the synthesis of mRNA has ceased (Table 2). The above results are, as a whole, in agreement with Mohr's hypothesis that P_{730} acts through the activation of potentially active genes. One point deserves further comment. In the case of anthocyanin synthesis in mustard, the mRNA is

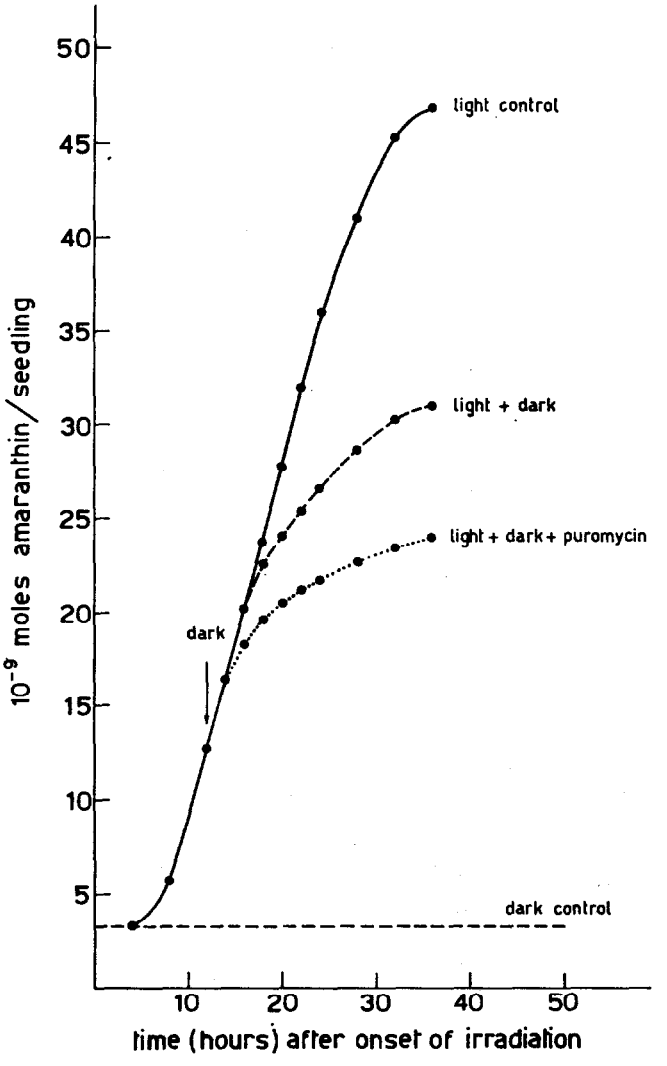

FIG. 3. EFFECT OF DARKENING (- - - -) AND DARKENING PLUS PUROMYCIN (·····) ON THE TIME COURSE OF AMARANTHIN ACCUMULATION IN CONTINUOUS LIGHT (——).

characterized by a short "life-time" and the dependence of actinomycin D inhibition on the time of its addition to the seedlings was explained by assuming that "actinomycin D in a suitable dose will block the formation of new kinds of mRNA but still permit the continued synthesis of mRNA already in production at the time the actinomycin is added".[3] In the case of amaranthin formation, since the mRNA involved in the synthesis of the pigment appears to be relatively stable, the time dependence of actinomycin D inhibition can be explained

without assuming that the antibiotic inhibits the potentially active genes but scarcely influences those already active at the moment of addition. It is impossible from our evidence to decide whether such differences in sensitivity to actinomycin D between active and potentially active genes exists also in *Amaranthus*.

EXPERIMENTAL

A cool-white fluorescent source giving an illumination of 5000 lx at the seedling level was used. Red and far-red sources were described previously.[6] Estimation of amaranthin has also been described.[6]

Plant Material

Seeds of *Amaranthus tricolor* were placed on two sheets of Whatman No. 1 filter paper moistened with tap water (14 ml) on 10 cm Petri dishes and held in complete darkness at constant temperature (28°).

Administration of Antibiotics

Dark-grown 2-day-old seedlings (100) of *A. tricolor*, after removal of the radicle, were incubated in 5-cm Petri dishes with a solution of actinomycin D (Calbiochem) or puromycin dihydrochloride (Nutritional Biochemicals Corporation) (10 ml) of appropriate concentration until the end of the experiments. All manipulations were carried out under dim green light. In preliminary experiments using intact seedlings, actinomycin D had a very low inhibitory effect, probably because it is not absorbed by the roots. The intensity of puromycin inhibition was approximately the same both in intact and rootless seedlings. Seedlings were not apparently damaged by 50 μg/ml puromycin. Six replicates were used in all experiments and each experiment was repeated at least eight times. The standard error was in each case 4% or less. Conc. of puromycin in Fig. 3 was 50 μg/ml.