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ACTION OF INHIBITORS OF PROTEIN AND NUCLEIC ACID SYNTHESIS ON LIGHT-DEPENDENT AND KINETIN-STIMULATED BETACYANIN SYNTHESIS

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Abstract—Light-stimulated amaranthin synthesis in *Amaranthus caudatus* seedlings is inhibited by chloramphenicol, puromycin, ethionine, cycloheximide, 2-thiouracil and actinomycin D On the other hand the kinetin-dependent pigment production can be blocked immediately only by cycloheximide and by actinomycin D, the other inhibitors are only active if the seedlings are preincubated with them No effect on light-enhanced and kinetin-influenced amaranthin formation was given by 5-fluorouracil, 8-azaadenine, 8-azaguanine and 5-fluorodeoxyuridine A small part of pigment is produced independently of protein and nucleic acid synthesis, especially in tyrosine-fed seedlings A working scheme is proposed, based on this and previously reported experiments, according to a hypothesis of Mohr

INTRODUCTION

THE POSTULATED mechanism for the light-induced production of cell sap soluble pigments are quite variable Several authors have attributed to the influence of light to an enhanced production of precursors, especially acetyl (malonyl) CoA for the ring A of flavonoid skeleton,[1] others demonstrated that phytochrome effects on flavonoid formation are dependent on protein (enzyme) and nucleic acid synthesis [2,3] Even kinetin acts on nucleic acid and protein metabolism and is able to induce enzymes, e g nitrate reductase[4] or tyramine-methyl pherase.[5] The present paper examines the influence of inhibitors of nucleic acid and protein synthesis in dependence of environmental conditions. Because betacyanin synthesis does not require acetyl (malonyl) CoA, the results provide important data for extended explanations of phytochrome and kinetin action.

RESULTS

Many inhibitors of protein and nucleic acid biosynthesis inhibit amaranthin formation, though the amount of inhibitor required for significant or complete suppression of pigment production are very different (Table 1). Some purine and pyrimidine derivatives have no effect, e g. 5-fluorouracil (10^{-7}–10^{-5} mol/l), 8-azaadenine (10^{-7}–10^{-5} mol/l), 8-azaguanine (10^{-7}–10^{-5} mol/l), 5-fluorodeoxyuridine (1–100 μg/ml) and 5-bromouracil (10^{-6}–10^{-3} mol/l) Further, the experiments demonstrate that many antibiotics and unnatural purine and pyrimidine derivatives, administered at the beginning of the light treatment, suppress only light-influenced amaranthin production, but not the kinetin-dependent amount With chloramphenicol (200 μg/ml), for instance, only the light-induced amaranthin production is inhibited, whereas the kinetin-dependent synthesis was not

[1] S B HENDRICKS and H A BORTHWICK, in *Chemistry and Biochemistry of Plant Pigments* (edited by T W GOODWIN), p 405, Academic Press, London–New York (1965)
[2] H A STAFFORD, *Plant Physiol* **41**, 953 (1966)
[3] H SCHERF and M H ZENK, *Z Pflanzenphysiol* **57**, 401 (1967)
[4] H BORRISS, *Wiss Z d Universität Rostock, Math -nat Reihe* **16**, 629 (1967)
[5] C E STEINHART, J D MANN and S H MUDD, *Plant Physiol* **39**, 1030 (1964)

TABLE 1 ACTION OF INHIBITORS OF PROTEIN AND NUCLEIC ACID SYNTHESIS ON AMARANTHIN SYNTHESIS (LIGHT-DEPENDENT SYNTHESIS)

Inhibitor	Significant inhibition (μmol/l)	Concentration giving ⩾ 80% Inhibition of light-dependent synthesis (μmol/l)
Protein synthesis		
Chloramphenicol	150	600
Puromycin	10	52 5
Ethionine	1	100
Cycloheximide	0 01	1
Nucleic acid synthesis		
2-Thiouracil	40	80
6-Azauracil	1000	—
Actinomycin D	4	20–40
For comparison, inhibition of tyrosinase		
Phenylthiocarbamide	1	30

blocked. An apparent compensation of kinetin and gibberellic acid on chloramphenicol inhibition of protein synthesis was demonstrated also in other cases [6] Though other authors could show an inhibition of kinetin effects by puromycin,[7] it was not observed in the amaranthin production of *Amaranthus* seedlings This holds true also of 2-thiouracil; an inhibition of its effect was only possible if the *Amaranthus* seedlings were preincubated with solutions of inhibitor In comparison, cycloheximide and actinomycin inhibit both the light-influenced and the kinetin-dependent pigment biosynthesis without preincubation

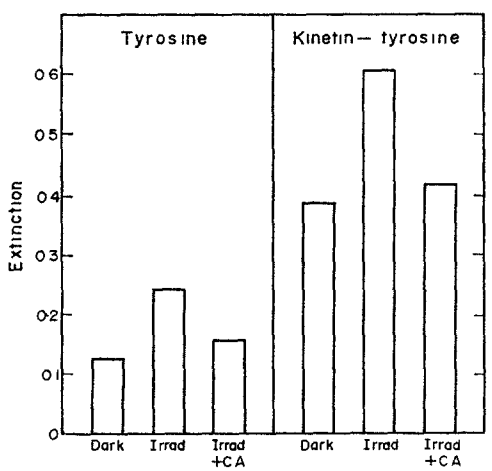

FIG 1 INHIBITION OF AMARANTHIN BIOSYNTHESIS BY CHLORAMPHENICOL (CA).

[6] O. N KULAJEWA and I P. WOROB'JEWA, *Fiziologiya rasteniy* (in Russian), **9**, 106 (1962)
[7] R. WOLLGIEHN and B. PARTHIER, *Phytochem* **3**, 241 (1964)

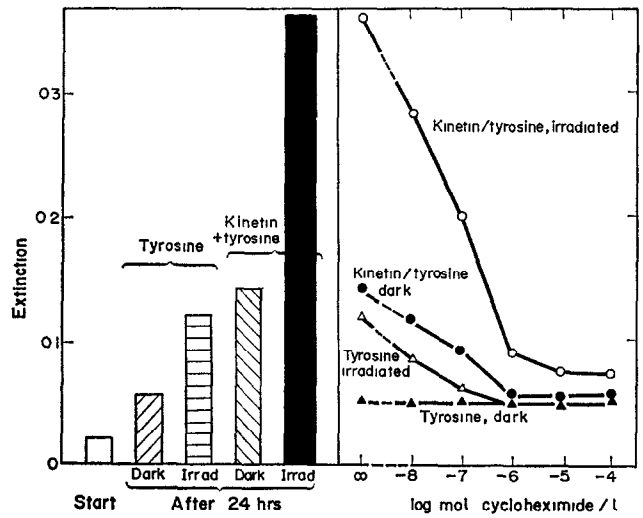

FIG 2 INHIBITION OF AMARANTHIN BIOSYNTHESIS BY CYCLOHEXIMIDE
(On the left side—Pigment production after 24 hr, on the right side—Inhibition by different doses of inhibitor)

(Figs 2 and 3). It is also recognizable that a small quantity of amaranthin is formed even in darkness especially if the seedlings were fed with the precursor tyrosine This may be due to small amounts of constitutive enzymes in the chain of betacyanin synthesis being present.

In every case, the effect of the inhibitors decreases in proportion to the time elapsed before irradiation of the seedlings occurred This is noticeable 4 hr after onset of light treatment. After 12 hr (chloramphenicol) or 16 hr (2-thiouracil, puromycin) inhibition proves impossible

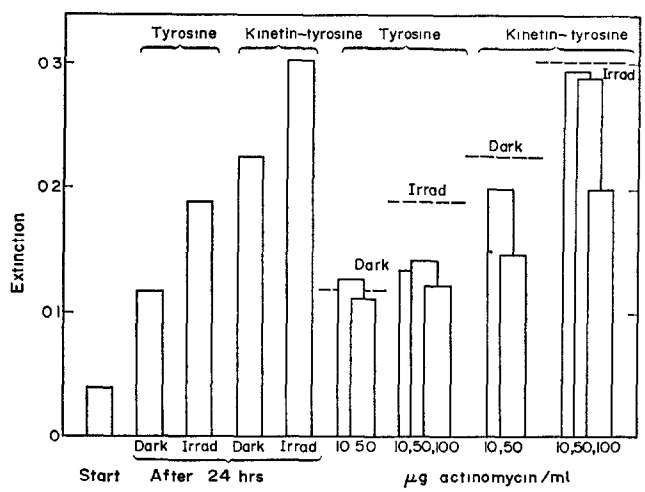

FIG 3 INHIBITION OF AMARANTHIN BIOSYNTHESIS BY ACTINOMYCIN
(On the left side—Pigment production after 24 hr, on the right side—Inhibition by different doses of inhibitor)

DISCUSSION

Referring to a hypothesis of Mohr[8] on photomorphogenic reactions, we propose a working scheme for explanations of our results and for further problems involved in the regulation of betacyanin synthesis (Fig 4) Amaranthin production in *Amaranthus caudatus* seedlings is regulated by the phytochrome system, by continuous far-red irradiation, by high energy blue light, and by kinetin [9] The correlations between light and kinetin seem to

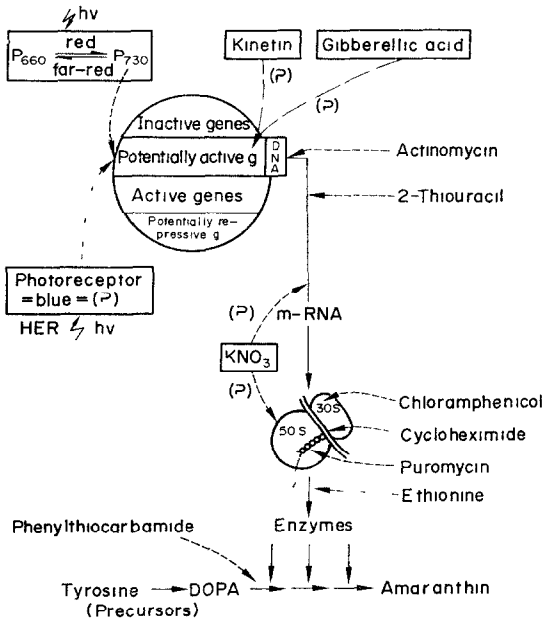

FIG 4 WORKING SCHEME

be additive. The question arises as to how the action of these exogenous factors is realized and correlated? Mohr stated that the phytochrome system may activate or derepress genes and induces in this way several enzymes for the biosynthetic chain According to this opinion, all the photoresponses > 550 nm are due exclusively to the phytochrome system [10] A further striking point of discussion is the involvement of the blue light action in betacyanin synthesis It is thought that the primary effect of blue high energy light might consist in the removal of repressors of the genes [3] Because phytochrome has an appreciable absorption in the blue region of the spectrum it is possible too that blue light should establish perhaps partly a P_{fr}-level intermediate between that achieved with either red or far-red irradiations But long-term treatments with high energy blue light seem to lead to a high-energy (HER) photoreaction which can be further regulated by the phytochrome system [3 10,11] All above mentioned results with inhibitors of protein and nucleic acid biosynthesis demonstrate that amaranthin is only formed in tissues which are not disturbed by these inhibitors. So the influence of phytochrome, high energy blue light and kinetin on amaranthin synthesis

[8] H MOHR, *Z Pflanzenphysiol* **54,** 63 (1966)
[9] K -H KOEHLER, *Phytochem* **11,** 133 (1972)
[10] H MOHR, *Natur Rundschau* **23,** 187 (1970)
[11] J W MCCLURE and K G WILSON, *Phytochem* **9,** 763 (1970)

seems to be realized via nucleic acid and protein (enzyme) synthesis In the scheme the action of different inhibitors—as demonstrated in this paper—is sketched; details for action of the different inhibitors are given in other papers [12,12a,13] Also the countereffect of gibberellic acid (GA_3) cannot be localized at present But from our experiments[12,12a] it is known that higher concentrations of gibberellic acid can diminish the promotion of kinetin.[14] Furthermore, we found a promoting effect of KNO_3 on amaranthin production, and it could be demonstrated that the influence of KNO_3 is inhibited by 2-thiouracil and chloramphenicol [15] Betacyanins are synthesized according to a hypothesis of Wyler *et al.*[16] from two molecules of DOPA At present, neither any single step nor the complete enzyme chain for betacyanin biosynthesis is known exactly But the inhibition with phenylthiocarbamide points to the plausibility of the suggested biosynthesis [12,12a] However, it is not possible at present to demonstrate how betacyanin synthesis is regulated by the above mentioned factors, i e which are the limiting enzymes and reactions. But the scheme presented may give suggestions for further work in this field

EXPERIMENTAL

Culture of plant material, extraction and estimation of amaranthin and light sources were described previously [9] For inhibitor experiments seedlings were placed in corresponding solutions of inhibitors for determined times

[12] K -H KOEHLER, *Habilitationsschrift Universität Greifswald* (1968), see also *Biolog Rundschau* **8**, 50 (1970)
[12a] M PIATELLI, M GIUDICI DE NICOLA and V CASTROGIOVANNI, *Phytochem* **9**, 785 (1970)
[13] J O SAZYKIN, *Antibiotics as Inhibitors of Biochemical Processes* (in Russian), Moscow (1968)
[14] K -H KOEHLER, *Biochemie Physiologie d Pflanzen* (in press)
[15] K.-H KOEHLER and D BIRNBAUM, *Biolog Zentralblatt* **89**, 201 (1970)
[16] H. WYLER, T J MABRY and A S DREIDING, *Helv chim Acta* **46**, 1745 (1964).

Key Word Index—Amaranthus caudatus, Amaranthaceae, betacyanin biosynthesis, kinetin, protein synthesis inhibitors, nucleic acid synthesis inhibitors