Photocontrol of Anthocyanin Synthesis

V. FURTHER EVIDENCE AGAINST THE INVOLVEMENT OF PHOTOSYNTHESIS IN HIGH IRRADIANCE REACTION ANTHOCYANIN SYNTHESIS OF YOUNG SEEDLINGS

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ABSTRACT

The apparatus of effectiveness and the synthesis of the only photoreceptor be disks leaf thethetic tor in. The action of the two antibiotics on anthocyanin synthesis is probably independent of the action of light. The results provide further evidence that the role played by photosynthesis in high irradiance reaction anthocyanin synthesis of young seedlings is only a minor one, if at all.

Studies of the action of light on the synthesis of anthocyanins (16) have been used quite extensively to gain some knowledge about the nature of theHIR3 photoreceptors. Most researchers agree that phytochrome is involved in HIR responses (2, 4, 6, 7, 9, 12, 18) and it has also been suggested that phytochrome may be the only photoreceptor involved, at least for HIR responses in the FR region (2, 7, 10, 12, 18). However, it has not been definitely proved that phytochrome is the only HIR photoreceptor and it has been suggested that other photoreactive systems may play a role in HIR responses (4, 13–15). There is some evidence for a contribution of photosynthesis to the synthesis of anthocyanins in green systems which have a functional photosynthetic apparatus, specifically apple skin sections and strawberry leaf disks (3, 4). Photosynthesis may play a role in the HIR anthocyanin synthesis of young, dark-grown seedlings exposed to continuous FR irradiation (13, 14). However, the latter suggestion is not supported by results obtained in various laboratories, including our own (5, 10–12, 19). The results submitted in this paper confirm and extend previous observations (11). One of the points in which we were specifically interested was to determine if the action of streptomycin on light-dependent anthocyanin synthesis would result in an alteration of the basic features of this response, that is, the R-FR reversibility and the relative effectiveness of different irradiance levels and of different spectral regions. Our interest is related to an assessment of the validity of the interpretation of in vivo inhibitor studies.

1 This research was partially supported by National Science Foundation Grants GB-35460 and BMS-74-19976 to A. L. M.
2 Present address: Division of Biology, SUNY at Stony Brook, N. Y. 11794.
3 Abbreviations: HIR: high irradiance reaction; FR: far red; R: red; B: blue; CWI: white; RI: a mixture of red and far red; DC: dark control; D: dark; L: light; STM: streptomycin.

MATERIALS AND METHODS

Seeds of cabbage (Brassica oleracea, cv. Red Acre) and tomato (Lycopersicon esculentum, cv. Beefsteak) were germinated and grown in darkness at 20 C, in Petri dishes, on two disks of No. 3 Whatman filter paper, moistened with distilled H2O or with the antibiotic solution. Lots of 30 and 75 seeds per dish were used for cabbage and tomato, respectively. Unless otherwise indicated, the light treatments were started 96 hr after sowing. The light treatments were given in growth chambers (Percival E-57) equipped with the light sources described in previous papers (8, 11). Temperature during the light treatments was 20 C. Dark controls were included in all experiments.

Anthocyanin and Chlorophyll Extraction and Measurement. Lots of 30 (cabbage) or 75 (tomato) seedlings each were extracted with 1% HCl in methanol (w/v) for 2 days, at 3 to 5 C, with continuous shaking. The extracts were clarified by filtration and their absorbances at 530 and 657 nm measured with a Gilford 300-N spectrophotometer. The anthocyanin content of the extracts is presented as A530 = 0.33 A657, a formula used to correct for the contribution of Chl and its degradation products in acid solution to the absorbance at 530 nm (11). The Chl content of the seedlings is presented as the A657 value of the acidic methanol extracts in Figure 2 and Table I. There is a good linear relationship between the absorbance of the acidic methanol extracts at 657 and the total Chl content of the seedlings as determined with extraction in 80% acetone and measurements according to Arnon (1); an A657 value of 0.5 corresponds to about 50 μg Chl/10 seedlings; the Arnon method was used to obtain the Chl data presented in Figures 1 and 3.

Measurements of O2 Exchange. Uptake and evolution of O2 were measured according to the manometric techniques described by Umbreit et al. (17), using a photosynthetic model Gilson respirometer. After equilibration, the O2 exchange of the seedlings placed in the manometric flasks was measured at 10-min intervals, first for 30 min in darkness, then for 30 min in light, using the light source of the Gilson respirometer, and again for 30 min in darkness. The readings used for computation of the rate of O2 exchange were those taken at the 20th and 30th min of each dark and light sequence. The results reported are the average of seven replicates.

RESULTS

The irradiance dependencies of anthocyanins and Chl production under continuous FR fall into different ranges (Fig. 1). Anthocyanin synthesis reaches about 90% saturation at an irradiance of 130 μW cm−2, and is fully saturated at an irradiance of 400 μW cm−2, there is no evidence for the saturation of Chl production within the ranges of FR irradiances used. The irradiance dependence of the a/b ratio follows closely the irradiation dependence of Chl production; at the irradiances that are most effective for anthocyanin synthesis, in terms of the ratio of
anthocyanin formed/dose applied, both the Chl content and the a/b ratio are quite low.

Large quantities of anthocyanins can be formed during a dark incubation period following exposure to continuous irradiation (Fig. 2). The synthesis of Chl under continuous FR proceeds at a much lower rate than that of anthocyanins, and there is a clear difference in the time course of anthocyanin and Chl under continuous R. Anthocyanin production under continuous R reaches saturation with a 9-hr irradiation, as shown by the experiments in which exposures of various durations are followed by a dark incubation period; but there is no evidence for saturation of Chl production with the irradiation times used.

Both STM and CAP enhance the synthesis of anthocyanins and inhibit the synthesis of Chl in cabbage and tomato seedlings (Table I). Streptomycin and CAP have no effect on the relative effectiveness of radiation in various spectral regions on the synthesis of anthocyanins in cabbage (Table I), and only a minor effect in tomato seedlings. The results obtained with tomato are not shown since they were similar to those obtained with cabbage. In tomato, the relative B-CWI-FR-R-Ri effectiveness ratios were 100:161:11:50:69 in water, 100:153:10:56:70 in STM, and 100:153:11:62:82 in CAP. Streptomycin and CAP do not affect the R-FR reversibility of anthocyanin production induced by a single, short irradiation (Table I).

There is a linear relationship between the increase of anthocyanins and the decrease of the Chl content brought about by various concentrations of CAP (Fig. 3).

Streptomycin does not affect the relative effectiveness of different irradiance levels upon anthocyanin synthesis under FR in cabbage, and under B in tomato (Table II); the reason for the choice of these two particular spectral regions is that FR is the most effective for anthocyanin synthesis in cabbage and B is the most effective for anthocyanin synthesis in tomato.

Streptomycin seems to have only a negligible effect on the O₂ uptake of cabbage seedlings and a strong inhibitory effect on O₂ evolution in light (Table III).

**DISCUSSION**

The results reported in this note and in previous ones (11, 19) show that STM and CAP inhibit the development of the photosynthetic apparatus and strongly enhance anthocyanin synthesis in young seedlings. The cause for the enhancement of anthocyanin production has not been established; it has been suggested (19) that the inhibition of the development of the photosynthetic apparatus may result in an increase of the pool size of one or more compounds needed for anthocyanin synthesis; this suggestion is supported, but not proven, by the results shown in Figure 3.

A reasonable interpretation of these results is that the contribution of photosynthesis to the HIR anthocyanin synthesis process of young seedlings is minimal, if at all. The interpretation of the results of inhibitor studies in vivo is not simple and requires a great deal of caution. One aspect of the results that gives us confidence in our interpretation is that the basic features (R-FR reversibility, irradiance dependence, and spectral sensitivity) of the responses are not affected by the antibiotics. The ratios of the levels of anthocyanin produced after a 5-min R and a 5-min R-5-min FR treatment are the same in water, STM, and CAP (Table I). The relative effectiveness of various spectral regions on anthocyanin production remains essentially the same in water, STM, and CAP (Table I). The relative effectiveness of different irradiance levels is essentially equal in water and in STM (Table II). The rate of respiration is not affected by STM (Table III); this result may be taken as an indication that the energetic demands of anthocyanin synthesis can be satisfied by respiration without an apparent need for the products of photosynthetic activity. The results of the experiments in which the antibiotics were not used corroborate the findings of the inhibitors studies: the irradiance dependence and the time course of Chl synthesis are quite different from those of anthocyanin synthesis (Figs. 1 and 2), and large quantities of anthocyanin can be formed before any considerable accumulation of Chl occurs. It has been reported recently that seedlings totally lacking photosynthetic capabilities, either due to a genetic lesion or to excision of the photosynthetic tissue, show increased rates of light-dependent anthocyanin formation (5). In general, it seems that the synthesis of Chl and the development of the photosynthetic apparatus may be detrimental to the formation of anthocyanins in young seedlings.

Enough evidence has been accumulated suggesting that the
Table I. Action of Streptomycin and Chloramphenicol on Synthesis of Anthocyanins and Chl in Cabbage Seedlings

Pigments were extracted 48 hr after the beginning of light treatments. Absorbance values of the anthocyanins produced in light were corrected by subtracting the absorbance values of the dark controls. Anthocyanins (Anth) as $A_{530} - 0.33A_{657}$. Chl as $A_{657}$.

<table>
<thead>
<tr>
<th>Light treatments</th>
<th>Water</th>
<th>STM (200 μg/ml)</th>
<th>CAP (10 μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anth</td>
<td>Chl</td>
<td>Anth</td>
<td>Chl</td>
</tr>
<tr>
<td>Dark Controls</td>
<td>0.24</td>
<td>0.02</td>
<td>0.35</td>
</tr>
<tr>
<td>Continuous B</td>
<td>0.86(88)$^1$</td>
<td>0.50</td>
<td>1.92(89)</td>
</tr>
<tr>
<td>Continuous CWI</td>
<td>1.16(118)</td>
<td>0.55</td>
<td>2.48(115)</td>
</tr>
<tr>
<td>Continuous FR</td>
<td>0.96(100)</td>
<td>0.06</td>
<td>2.16(100)</td>
</tr>
<tr>
<td>Continuous R</td>
<td>0.44(45)</td>
<td>0.41</td>
<td>1.00(46)</td>
</tr>
<tr>
<td>Continuous RI</td>
<td>0.84(86)</td>
<td>0.50</td>
<td>1.75(81)</td>
</tr>
<tr>
<td>5 min R</td>
<td>0.15(15)</td>
<td>---</td>
<td>0.32(15)</td>
</tr>
<tr>
<td>5 min R - 5 min FR</td>
<td>0.07(7)</td>
<td>---</td>
<td>0.16(7)</td>
</tr>
</tbody>
</table>

1. Numbers in parentheses are % anthocyanin production (FR = 100)

Table II. Action of Streptomycin on Synthesis of Anthocyanin in Cabbage and Tomato Seedlings Exposed to Continuous Irradiation

Anthocyanins were extracted 48 hr after the beginning of light treatments.

<table>
<thead>
<tr>
<th>Cabbage</th>
<th>Tomato</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiance ($FR$, $μW$ cm$^{-2}$)</td>
<td>$A_{530} - 0.33A_{657}$ (Water, STM$^*$)</td>
</tr>
<tr>
<td>480</td>
<td>1.45(100)</td>
</tr>
<tr>
<td>240</td>
<td>1.25(86)</td>
</tr>
<tr>
<td>120</td>
<td>1.09(75)</td>
</tr>
<tr>
<td>60</td>
<td>0.91(63)</td>
</tr>
<tr>
<td>DC</td>
<td>0.24</td>
</tr>
</tbody>
</table>

* STM = streptomycin, 200 μg/ml

The contribution of photosynthesis to an HIR anthocyanin synthesis of young seedlings is minimal, if at all (5, 11, 12). On the basis of this evidence, we would be inclined to conclude that the involvement of photosynthesis in the HIR is not general, but of the HIR, even though there is some evidence for such an involvement in some adult, green systems (3, 4). Perhaps the time has come for a detailed study directed to investigate the exact nature of the relationships between photosynthesis and the
Fig. 3. Action of chloramphenicol on synthesis of anthocyanin in cabbage seedlings exposed to a 48-hr irradiation with a mixture of R and FR. Numbers in the circles are concentrations of chloramphenicol in µg/ml; (○): anthocyanin versus Chl a; (□): anthocyanin versus Chl(a+b).

Table III. Action of Streptomycin on Oxygen Exchange of Cabbage Seedlings

<table>
<thead>
<tr>
<th>Light</th>
<th>Oxygen Exchange (µl O₂/10 min/10 seedlings)¹</th>
<th>Water</th>
<th>Streptomycin (200µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D</td>
<td>L²</td>
</tr>
<tr>
<td>DC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hr FR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>48 hr FR</td>
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</tbody>
</table>


HIR in the systems which have a functional photosynthetic apparatus. We are aware that the results reported in this and other papers (5, 11) are of a negative type, and have probably gone as far as possible in providing a contribution to the understanding of HIR phenomena; they do not provide a direct clue toward the identification of the HIR photoreceptors; but they have been useful insofar as they have contributed to the elimination of certain possibilities and are consistent with the hypotheses (2, 7, 10, 12, 18) suggesting that phytochrome may be the only photoreceptor involved in the HIR.

LITERATURE CITED