Phytochrome-mediated Induction of Phenylalanine Ammonia-Lyase in Mustard Seedlings

A CONTRIBUTION TO ELIMINATE SOME MISCONCEPTIONS

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ABSTRACT

The present report shows that the effect of red and far red light on the synthesis of phenylalanine ammonia-lyase can be ascribed to the action of phytochrome Pfr. This is true not only for short term irradiation but also for the action of continuous far red light. In the latter case, the model worked out by Hartmann implicating some excited species of phytochrome has to be taken into account.

It is generally accepted that the following operational criteria establish the involvement of phytochrome in a light-mediated response: an induction effect by a brief irradiation with red light can be fully reversed by a subsequent saturating pulse of far red light. However, the quantitative relationship between the concentration of Pfr, (Pfr), and the extent of the response, AM, must be determined individually for every photoreceptor. This is immediately apparent if we take into account that in some systems (e.g., cotyledons of the mustard seedling) Pfr can control the synthesis of lipoxygenase through a threshold mechanism (13), whereas in the case of anthocyanin synthesis, no threshold mechanism can be detected (9). Using data from in vivo spectrophotometric phytochrome measurements, the quantitative elaboration of the function AM = f ([Pfr]) is in principle feasible for any particular photoreceptor. Hitherto, it was only in the case of Pfr-mediated repression of lipoxygenase synthesis (13) that a clear-cut function of this type could be formulated. In other cases, the correlation between AM and (Pfr) is not equally obvious (e.g., 9), and “non-rational” relationships, i.e., “phytochrome paradoxes” (8), have been reported. One contributing factor in these paradoxes might be that kinetically different phytochrome populations (3), so far not distinguishable by the usual spectrophotometric measurements, are involved in photomorphogenetic responses.

In this paper we wish to stress the following point: the spectrophotometrically measurable Pfr represents Pfr in the ground state (nonexcited). In the case of light-pulse responses, the above mentioned function might more correctly be written: \[ \Delta M = f ([Pfr_{ground state}]), \] wherein \([Pfr_{ground state}]\) is the concentration of Pfr established immediately after termination of a brief irradiation.

Continuous irradiation with far red light leads to strong photomorphogenetic effects (6, 10), the extent of which is a function of the incident quantum flux density (7, 16). This type of response has been called “high intensity reaction”. According to Hartmann (4, 5, 7), the high intensity reaction is attributable to the phytochrome system. Indeed, we have obtained no data with the mustard seedling which could not be reconciled with the hypothesis which has been advanced and elaborated by Hartmann (4), and according to which the morphogenetic effects of continuous light of wavelengths greater than 550 nm can be attributed to phytochrome (9). Hartmann (4, 7) has explained the irradiance dependence by the hypothesis that Pfr can act from some excited state, Pfr*, which is more active than Pfr in the ground state. In brief: standard continuous far red light (cf. “Materials and Methods”) maintains a relatively low, but highly effective level of Pfr (by this term we do not discriminate between Pfr_{ground state} and Pfr* in the tissue). The extent of the response (effect) is a logarithmic function of irradiance (quantum flux density), at least in the middle part of the irradiance-effect curve (4, 7, 16). While speculations about the nature of the excited state are in print (4), this state can at present not be measured directly. Therefore, it is at present not possible to elaborate the function \[ \Delta M = f ([Pfr*]), \] wherein \(\Delta M\) is the extent of the response (in general a rate) and [Pfr*] is a steady state concentration of the excited species. It follows from these considerations that the hypothesis (that Pfr is the effector of the high intensity reaction) cannot be tested by simply comparing spectrophotometric phytochrome data with the effects of continuous far red light treatment. We have summarized these arguments because the distinction between Pfr_{ground state} and Pfr* has not been considered to be relevant in a recent, critical paper by Bellini and Hillman (2).

These authors summarize their work as follows: “In seedlings of Raphanus (radish) and Sinapis (mustard), irradiation for 6 hr with far red light significantly increases the extractable activity of PAL by the end of the light period. A schedule of 10 min red light—110 min darkness—10 min red—110 min darkness—10 min red—110 min darkness has no effect as compared to dark controls. However, the red light program maintains a level of Pfr always measurable by in vivo spectrophotometry during the 6 hr experimental period. We conclude that the far red effect on this enzyme and for this specific

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2 Although phytochrome might be compartmentalized, the term “concentration” is used since in the usual spectrophotometric measurements it is the total phytochrome concentration which is determined.

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* Abbreviation: PAL: phenylalanine ammonia-lyase.
material cannot be explained solely by formation and maintenance of Pfr."

We show in the present paper that as little as one 5 min irradiation of red light does, in fact, induce PAL formation, and that the operational criteria for the involvement of Pfr ground state in the induction of this response are fulfilled. We further show that the effect of continuous far red light on PAL formation is characterized by the usual criteria of the high intensity reaction and thus can be understood on the basis of Pfr* in terms of Hartmann's hypothesis (4). Thus the data reported in the present paper are not consistent with the conclusions of Bellini and Hillman (2).

MATERIALS AND METHODS

Seeds of Sinapis alba L. (obtained in 1968 from Becker, Heilbron) were selected and germinated according to standard techniques developed in this laboratory (11). The seedlings were grown at 25.0 ± 0.2 C for 48 hr in the dark before light treatment was started. For irradiation the standard red (12) and far red (11) sources were used at an irradiance of 67.5 \( \mu \text{W cm}^{-2} \pm 10\% \) (red) and 350 \( \mu \text{W cm}^{-2} \pm 10\% \) (far red) at 25.0 ± 0.2 C. In the experiments of Figure 2 the far red irradiance was reduced by approximately covering the source.

The standard methods of extraction and assay of PAL described elsewhere (14) were modified as follows: 40 pairs of cotyledons were dissected at 2 C and homogenized at 0 C with 6.0 ml 0.1 M borate buffer, pH 8.8. After centrifugation, a 4.5-ml aliquot of the supernatant was freed of low molecular weight components by low speed centrifugation through a 14-x 60-mm column of Sephadex G-25 (fine) equilibrated with the borate buffer. Before application of the enzyme extract the liquid in the void volume was removed by centrifugation. This treatment yielded a highly ultraviolet transparent extract at the original enzyme concentration which could be assayed without further addition of buffer. The test mixture contained 2.5 ml of enzyme extract and 0.5 ml 0.1 M phenylalanine. This procedure gives a 6-fold increase in sensitivity over the usual procedure (14). The values presented are means of four to eight independent experiments. Standard errors are indicated by vertical bars when exceeding the size of the symbols.

RESULTS AND DISCUSSION

Figure 1 shows that one 5 min irradiation with red or far red light, applied 48 hr after sowing (time zero in the figure),

![Figure 1](image1.png)

**Figure 1.** Increase of PAL activity under continuous far red light, or following a brief irradiation (5 min) with red or far red light at time zero.

![Figure 2](image2.png)

**Figure 2.** Dependence on irradiance of PAL synthesis. Irradiance of standard far red light = 100%. Enzyme extraction: 3 hr after onset of continuous far red light (cf. Fig. 1). will induce enzyme synthesis in the mustard cotyledons. (The term "synthesis" has been justified previously with respect to PAL [15]). Similar, but somewhat lower, effects of the light treatments were obtained with 36-hr-old seedlings. The discrepancy between Bellini and Hillman's results and those in the present paper might be due to their use of a less sensitive method of PAL assay at a less light-sensitive stage of seedling development. Table I shows that the operational criteria for the involvement of phytochrome in this induced synthesis are fulfilled. The relatively strong effect of 5 min of far red light indicates that PAL formation is very sensitive towards Pfr ground state. The enzyme activity isolated from cotyledons of dark-grown seedlings is low, and increases only slightly during the experimental period. Clearly, the statement of Bellini and Hillman (2) that brief treatments with red light have no effect on PAL activity in mustard seedlings as compared to dark controls, is not correct. Our present results confirm earlier work (17) where several brief treatments with red (or far red) light were applied. Promotion of PAL synthesis by a single brief red light treatment and its subsequent reversion by far red has been shown also in pea seedlings (1).

Continuous standard far red light (cf. "Materials and Methods") mediates a strong increase of PAL synthesis (Fig. 1). The lag-phase after the onset of light is probably the same as in the case of a brief light treatment. Since the photo-stationary state of the pigment characteristic of this far red light is established by the standard far red light rapidly (on the order of 1 min), we assume that from the very beginning a nearly stationary

<table>
<thead>
<tr>
<th>Light Treatment</th>
<th>Enzyme Activity</th>
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<tbody>
<tr>
<td>48 Hr dark</td>
<td>4.1 ± 0.3</td>
</tr>
<tr>
<td>52 Hr dark</td>
<td>5.2 ± 0.5</td>
</tr>
<tr>
<td>48 Hr dark + 5 min red + 4 hr dark</td>
<td>13.6 ± 0.6</td>
</tr>
<tr>
<td>48 Hr dark + 5 min far red + 4 hr dark</td>
<td>10.8 ± 0.6</td>
</tr>
<tr>
<td>48 Hr dark + 5 min red + 5 min far red + 4 hr dark</td>
<td>10.4 ± 0.6</td>
</tr>
<tr>
<td>48 Hr dark + 5 min far red + 5 min red + 4 hr dark</td>
<td>14.3 ± 0.8</td>
</tr>
</tbody>
</table>

Table I. Reversion Experiments which Demonstrate that the Operational Criteria for the Involvement of Phytochrome in Light-Mediated Increase of PAL are Fulfilled.
concentration of Pfr* is acting in the cotyledons. The dependency on irradiance of the effect of continuous far red light is shown in Figure 2. Irrespective of the interpretation of the irradiance dependency in terms of molecular physics, we conclude that the response of the PAL-producing system in the mustard cotyledons can be explained in terms of the model worked out by Hartmann (4, 5, 7). There is no reason to conclude (2) that the far red effect on PAL synthesis in the mustard seedling "cannot be explained solely by formation and maintenance of Pfr." However, it is necessary to discriminate between Pfr(ground state) and Pfr* and to ascribe most of the effect of continuous far red light to the action of Pfr*. We agree, of course, that the interpretation of Pfr* in physical terms is still an open question; however, the experimental evidence that the action of continuous far red light is due in some way to Pfr has been convincingly presented by Hartmann (4–7).

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LITERATURE CITED