Short Communication

Responses of Avena and Pisum Tissues to Phytochrome Conversion by Red Light

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Since the phytochrome in seedlings grown in darkness is entirely in the red-absorbing (Pr) form, it is reasonable to suppose that the morphogenetic effects of red light are due to formation of the far-red-absorbing (Pfr) form (4). This hypothesis can be studied by briefly illuminating dark-grown tissues, replacing them in darkness, and determining the relationship between the percentage of the total phytochrome initially converted to Pfr and the subsequent physiological response. It is important to note, however, that there are 2 distinct ways of establishing a given percentage conversion to Pfr. The most obvious is to vary the dosage of essentially pure red light. The other is to give saturating exposures to sources emitting both red and far-red in ratios maintaining various photostationary states (2, 3, 4). One might expect results obtained by the 2 methods to agree, but this expectation has not so far been carefully tested.

Reports that the growth responses of segments of both Pisum stems (2) and Avena coleoptiles (3) require a substantial phytochrome conversion (at least 50% Pfr) for saturation depend on photostationary state methods, though with some indications that in Pisum the results with red light would fall in the same range. On the other hand, the effects of red light pretreatment on Zea phototropism saturate at least 2 orders of magnitude below the energy required to give detectable phytochrome conversion, though the phenomenon is far-red-reversible (1 and Briggs, private communication). Since all far-red filters leave at least 1% Pfr (2, 3, 4) the Zea results represent a complete contradiction between the results of the red dosage and photostationary state methods. Hence, it seemed desirable to re-examine the Pisum and Avena results previously obtained, this time by the red dosage method.

With the exception of the red light source, all materials and procedures were as described previously (2, 3). This includes the convention on calculating percentage Pfr, in which a correction is made for incomplete conversion by far-red, but not for incomplete conversion by red. The red source was a bank of incandescent spotlights behind 10 cm of water, 3 mm thickness of red plexiglas (Rohm and Haas 2444) and Corning Filter 494, and thus substantially the same as source III-E previously described (2). Intensity was varied 10-fold with a variable transformer and exposure times ranged from 6 seconds to 4 minutes. The total energy of red light given by a 2-minute exposure at intensity 1.0 is described as relative red 100 and the other energy levels are described accordingly.

A calibration in terms of the exposures required to bring about a given phytochrome conversion was accomplished by exposing tissue samples and then assaying in the usual manner. There was no significant difference between the conversions achieved in the Pisum or Avena tissues employed. All values quoted are means of at least 6 samples assayed on at least 3 separate occasions. Exposures of relative red 100 and over gave Pfr levels of 92 to 100%, while exposures of relative red 5 gave roughly 17% (range 15–20) Pfr. Exposures of relative red 0.5 gave a level of Pfr not readily distinguishable from the standard far-red saturation, which is to say less than about 5% (2). With the times and intensities used, phytochrome conversion exhibited reciprocity. For example 4 minutes at intensity 0.1 and 24 seconds at intensity 1.0 (both giving relative red 20) each gave 40 to 47% Pfr.

With the red source thus calibrated, and after some orienting observations, the effects of relative red 0.5, 5 and 100, giving undetectable, 17 and 92 to 100% Pfr, respectively, were compared in a series of experiments designed to detect a phenomenon like that in Zea if it should exist in these systems. The results are summarized in figure 1, in which the effects of the 2 lower energies in each experiment are presented as percentages of the effects of the relative red 100 level in that experiment for each tissue. The figure thus summarizes all 10 experiments, including 3 each on Pisum and Avena alone and 4 in which both tissues were incubated and exposed in the very same beakers. All segments were initially 5.0 mm long and the mean response to relative red 100 for all experiments was, in Pisum, an elongation inhibition of 1.7 mm compared to the dark controls, and, for Avena, a promotion of 1.13 mm compared to the dark controls.

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Fig. 1. Responses of segments of *Avena* coleoptiles and *Pisum* stems to red light as percentages of their responses to a dose energy (relative red 100) giving essentially complete conversion of Pr to Pfr. Each point is from a single experiment and represents a comparison between means based on at least 30 segments each. Experiments A-6, 7, 8; P-228, 29, 31; AP-2, 4, 5, 7.

Relative red 5, giving an average 17% Pfr, elicited roughly 35% of the maximum response in *Pisum* and about 70% of the maximum response in *Avena*. The lower energy level, 0.1 of the preceding, gave about 20% of the maximum response in both *Pisum* and *Avena*. The results for both tissues are completely consistent with those previously obtained by the photostationary state method. Interpolation in the data already published would lead one to expect, from the 17% Pfr level, roughly 48% of the maximum effect in *Pisum* (2) and roughly 62% in *Avena* (3). The actual means of 35 and 70%, respectively, are as close as can reasonably be expected given the great variability in all the data. The results for the lower energy level also agree well on the reasonable assumption that the Pfr level produced is just below that caused by far-red radiation alone. Thus it is as clear from these data as it was from the photostationary state work that a substantial proportion of the detectable phytochrome must be initially converted in order to saturate these growth responses.

The paradoxical results in *Zea* (1) have been used to suggest that the bulk of the phytochrome, that detected spectrophotometrically, is not connected to an active site and that a very small active fraction may be so oriented as to be far more sensitive to conversion by low energies. A comparable segregation of active and bulk phytochrome, though one requiring differences in reversion rate and not in sensitivity to light, has been proposed for *Pisum* on the basis of far-red reversals obtainable in tissues without detectable Pfr (2). The results reported here contribute no further information on either type of contradiction, but they do show that the "Zea paradox" is not demonstrable in the growth of either *Pisum* stem or *Avena* coleoptile tissue. Since the *Avena* system was previously shown to lack the contradiction observed in *Pisum*, it is so far the only system in which no serious anomaly has been found in the relationship between Pfr and growth response (3).

In summary: For an initial brief illumination to saturate the subsequent growth response in excised segments of either etiolated *Pisum* stems or *Avena* coleoptiles, it must convert a substantial proportion of the phytochrome to Pfr. In both these materials, the results of experiments conducted with photostationary state illuminations are consistent with those obtained with various dosages of pure red. There is no evidence in either tissue for maximal or near-maximal effects of dosages too low to give spectrophotometrically detectable phytochrome conversions.

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Literature Cited