Short Communication

Response of Pea Stem Tissues With Different Phytochrome Contents to Red Light Dosage

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Partially de-etiolated Pisum stem segment tissues with a 4-fold difference in spectrophotometrically measurable phytochrome react in the same way to the same percentage conversion of phytochrome to the PFR form (1). These results are consistent with the theory that the amount of physiologically active phytochrome is so low that it is spectrophotometrically unmeasurable, while the bulk of the phytochrome is physiologically inactive and quantitatively variable. If this theory is correct, it is nevertheless possible that the bulk form functions as an accessory pigment by absorbing light and transferring the energy to the active centers which are then able to cause a physiological response. However, the results cited above were not done in a manner that would detect such an effect if it were present. There are 2 methods of establishing various percentages of PFR: in the photostationary state technique, sources of mixed red and far-red light are given to saturation, and the final pigment ratio is dependent on the quality of the light mixture. The alternative (red dosage) method employs very short exposures to pure red or far-red light so that the amount of pigment converted is limited by the incident energy. The latter method is generally much less convenient, and it has been shown with etiolated Pisum and Avena tissues, there is no significant difference in response between these 2 methods (3). However, the results cited above with de-etiolated tissue used the photostationary state method in which saturation of the pigment system would mask the functioning of an accessory pigment by allowing a minimal phytochrome content to be as effective as the complete system. For this reason, attempts were made to repeat these experiments with the red dosage method.

With the exception of certain aspects of the pre-treatments, all procedures and materials were as previously described (1). The phytochrome content of pea plants was varied by exposure to 16 hr of either 3 min of red light every 2 hr (R), or red light every 2 hr plus continuous low intensity far-red light (RF), or continuous darkness (D). All plants were then exposed to 15 min of far-red and then left in darkness for 5 hr before 10 mm apical segments were cut for the growth assay. In the previous work, the plants were given the same pretreatment except that only the R and RF plants received a terminal far-red exposure, and then all plants were sectioned immediately. The red light source used for photoconversion (3) gave 0.40 μW at 660 nm and 0.014 μW at 730 nm as measured with an Asco Model 3051 spectroradiometer. This energy level, arbitrarily called red intensity 1.0, could be varied with a rheostat. Since total energy is the product of the exposure time (seconds) times the red intensity factor, a dose of 30 is 30 sec of red intensity 1.0 or 5 min of red intensity 0.1. As usual, the spectrophotometrically assayable phytochrome was not corrected for incomplete conversion of PR to PFR (3).

Calibration of the red source to determine the dose needed to convert a given percentage of phytochrome was performed on completely dark-grown samples. Iced 5 mm pea segments with hooks were suspended in water and pretreated with 10 min of red light to photosaturate protochlorophyll conversion. After far-red reversal, the segments were exposed to red intensity 1.0 for different intervals, then packed in cells for measurement. Using the means of 6 samples assayed on at least 3 separate occasions, exposures of 0, 10, 30, and 60 sec to red intensity 1.0 yielded conversion of 0, 37, 77, and 96 % of the PR, respectively. A single determination confirmed that reciprocity holds since the same doses using red intensity 0.1 produced 0, 36, 80, and 93 % conversion. Interpolation of a log plot of dose vs. percent PR remaining showed that a dose of 5 converted 22 %, so doses of 0, 5, 10, and 30 were used since they converted amounts of phytochrome similar to those established by the photostationary state filters used in the previous work.

Since the calibration was performed on dark-grown tissue, it was necessary to show that light-
pretreated tissue also responded in the same way. The calibration methods used are time-consuming since each sample must be exposed to light, packed, and then the initial conversion and the total phytochrome determined. The methods of Purves and Briggs (5) although not suitable for the initial calibration of light sources, were preferable for the comparison. The same sample is used for the entire curve rather than separate samples for each point, and its $\Delta$OD is noted after each brief irradiation with the internal source until saturation occurs. When tissues from R, RF, and D pretreated plants were measured, no differences in the dose response curves were found, confirming earlier results (2). Therefore, any given dose produces the same percentage of P$_{FR}$ independently of the tissue pretreatment.

For the experiments themselves, lots of 15 10-mm segments from pretreated peas were cut, suspended in buffer, exposed to various doses of red intensity 1.0, placed in darkness for 20 hr, and then measured. In Fig. 1, the percentage increase in length is plotted against the percentage P$_{FR}$ established for each set of plants. The graph summarizes 4 experiments and each point is the average of duplicate lots. When these 3 figures are superimposed (Fig. 2, left), it is clear that tissue from the R and RF pretreated plants react in the same way. Since the RF tissue contains 4 times more measurable phytochrome than does the R treated tissue, the sections apparently respond not to various quantities of phytochrome, but rather to the percentage in each form (1). The etiolated tissue (D) contains levels of phytochrome similar to the RF plants, although their responses to light are different. This difference is presumably due to the decreased sensitivity of de-etiolated tissues to phytochrome action (2).

The photostationary state data published earlier (1) were presented with the elongation caused by 5% phytochrome conversion set at 100%. Since the 5% value, requiring less than a sec of red light, was omitted here, the published data were recalculated by setting the dark elongation at 100% and are presented in Fig. 2 (right). Comparison of Fig. 2 (left) and 2 (right) shows that R and RF treated tissues respond to various phytochrome levels in the same way even though the pigment levels were established in very different manners. Since the accessory pigment theory predicts that R pretreated tissue should respond less than the RF tissue to energy-limited percentages of P$_{FR}$, the superimposibility of both sets of data discounts the theory. Note that the dark controls of the 2 sets are not identical due to the different procedures used. Those in Fig. 2 (right) were never exposed to light, while those of the current experiments (Fig. 2, left) were given far-red treatment at the end of the dark period, followed by 5 hr of darkness. Hence the D tissue here is slightly de-etiolated, and responds more like the light-treated tissue.

Since the results obtained here with the red dosage technique confirm those obtained with photostationary state methods (1, 2), it seems unlikely that the phytochrome eliminated by P$_{FR}$ "destruction" (4) is acting as an accessory pigment.

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Literature Cited


