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In Vivo Phytochrome Reversion in Immature Tissue of the Alaska Pea Seedling[1]

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JAMES A. MCARTHUR[2] AND WINSLOW R. BRIGGS
Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

ABSTRACT

Reversion of far red-absorbing phytochrome to red-absorbing phytochrome without phytochrome destruction (that is, without loss of absorbancy and photoreversibility) occurs in the following tissues of etiolated Alaska pea seedlings (*Pisum sativum* L.): young radicles (24 hours after start of imbibition), young epicotyls (48 hours after start of imbibition), and the juvenile region of the epicotyl immediately subjacent to the plumule in older epicotyls. Reversion occurs rapidly in the dark during the first 30 minutes following initial phototransformation of red-absorbing phytochrome to far red-absorbing phytochrome. If these tissues are illuminated continuously with red light for 30 minutes, the total amount of phytochrome remains unchanged. Beyond 30 minutes after a single phototransformation or after the start of continuous red irradiation, phytochrome destruction commences. In young radicles, sodium azide inhibits this destruction, but does not affect reversion. In older tissues in which far red-absorbing phytochrome destruction begins immediately upon phototransformation, strong evidence for simultaneous far red-absorbing phytochrome reversion is obtained from comparison of far red-absorbing phytochrome loss in the dark following a single phototransformation with far red-absorbing phytochrome loss under continuous red light.

Though phytochrome is involved in a wide range of physiological reactions in plants, examinations to date have failed to elucidate the relationship, if any, between the dark reactions of the far red-absorbing form of the pigment, Pfr, and physiological response. The two dark reactions studied thus far are Pfr reversion to Pr, the red-absorbing form of the pigment, and destruction, namely loss of absorbancy and photoreversibility.

Early observations by Butler *et al.* (8) suggested that reversion occurred *in vivo* in tissues of dark-grown corn seedlings and in light-grown cauliflower florets. The apparent dark reversion of Pfr in corn (but not cauliflower) was subsequently shown to be an artifact arising from overlapping absorption at 660 nm both by Pr and Pfr (7). This overlap prevents complete phototransformation of Pr to Pfr. Taylor (23) has shown Pfr reversion *in vitro* for phytochrome from many different higher plants. *In vivo* reversion has been most clearly detected in the receptacle of *Cynara scolymus* (14), in cauliflower florets (8, 14), and in tissue cultures of wild carrot, *Daucus carota* (24). The kinetics of Pfr loss and total photoreversibility loss have been interpreted to suggest Pfr reversion in tissue from the taproot of *Pastinaca sativa* (19) and tissue from several dicotyledonous seedlings (16, 17). Physiological studies a number of years ago implicated Pfr reversion in the flowering process (2, 3) and in the germination of lettuce seeds (4).

Butler and Lane (7) used CO, N_2, NaN_3, and KCN, all of which inhibited Pfr destruction, to show an absence of Pfr reversion in maize tissues. Subsequent work by Furuya *et al.* (13) used metal-complexing and sulfhydryl-binding reagents to show that Pfr destruction is an oxidative metal-dependent process. When these workers blocked destruction, they found no evidence for *in vivo* reversion in the monocotyledonous tissues examined. Other workers (10, 21) deduced an absence of Pfr reversion in corn seedlings from kinetic considerations. Furuya and Hillman (11) used similar kinetic considerations to suggest the occurrence of Pfr reversion in tissue of Alaska pea seedlings. These and other findings are thoroughly reviewed by Hillman (15). Kendrick and Frankland (18) have recently showed an absence of *in vivo* reversion in a dicot, *Amaranthus caudatus*.

The present paper presents spectrophotometric evidence for rapid reversion of Pfr to Pr in the absence of any measurable Pfr destruction in immature tissues of etiolated pea seedlings. This reversion occurs during the 30 min immediately following a single red light irradiation. Evidence for reversion in older tissues showing simultaneous destruction is also presented.

MATERIALS AND METHODS

Peas (*Pisum sativum* L. cv. Alaska) were germinated and grown in darkness at 25 C and 85% relative humidity as described elsewhere (20). Samples were prepared for spectrophotometric observations under the same conditions, and all manipulations were carried out under dim green light. For a given experiment, plant parts were excised and divided into samples of equal weight and number of plant parts. Control samples in each experiment were chilled for 1 hr on ice before any light treatment, and then were kept on ice for the duration of the experiment.

Red light was obtained with cool white fluorescent bulbs and a 3 mm Plexiglas red filter (Rohm and Haas, No. 2423). The light intensity at the level of the tissue, as determined through a 5-cm water filter, was about 300 ergs cm^{-2} sec^{-1}. Correction for infrared wavelengths was obtained using a Corning 1-69 glass filter. Measurement was made with an Eppley thermopile and a Hewlett-Packard microvoltmeter as described elsewhere (21). When samples received continuous red light, they were prepared under the red light source and then placed into a chilled cuvet

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[2] Present address: Department of Botany, North Carolina State University, Raleigh, N. C. 27607.

for examination in a Ratiospect R-2 difference spectrophotometer (20).

Examination of chilled control samples in several experiments showed that the red light source mentioned above converted approximately 90% of the total phytochrome actually measurable in the Ratiospect. Since the precise photostationary equilibrium for pea phytochrome following saturating red irradiation is not known, the uncorrected directly measured photoreversibility of the chilled controls in the Ratiospect was arbitrarily used at 100%, and all other data calculated against this standard. The procedure for phytochrome measurement in the Ratiospect is described elsewhere (6).

RESULTS

Phytochrome appearance and distribution in the very young dark-grown pea seedling has been described elsewhere (20). In examining the effect of saturating amounts of red light upon phytochrome disappearance and Pfr loss at several different stages of seedling development, the dark reactions of older seedlings were found to be similar to those previously reported (11). However, the relationship between Pfr reversion and destruction in very young pea tissue was quite different.

Dark Reactions in Plant Parts of Different Ages. During the first 30 min after a single saturating dose of red light, there was virtually no change in the total amount of phytochrome in radicles less than 0.6 cm in length, while total phytochrome declined significantly in somewhat longer radicles. In both cases, however, there was rapid disappearance of Pfr, suggesting that, at least in the shorter radicles, clear reversion of Pfr to Pr was taking place (Fig. 1). Similarly, reversion could be detected in epicotyls 0.6 to 0.8 cm in length in the absence of significant Pfr destruction (Fig. 2), at least for the first 30 min following saturating red irradiation, while in older epicotyls (Fig. 3), total phytochrome begins declining immediately following the red light treatment. In even smaller epicotyls (0.4 cm), reversion in the absence of destruction is equally clear for the first half-hour after red light treatment, but the rate of reversion is somewhat slower than in older epicotyls. Finally, the same phenomenon may be observed in juvenile regions of older epicotyls (2–3 cm). Although in more basal tissue of these epicotyls disappearance of total phytochrome

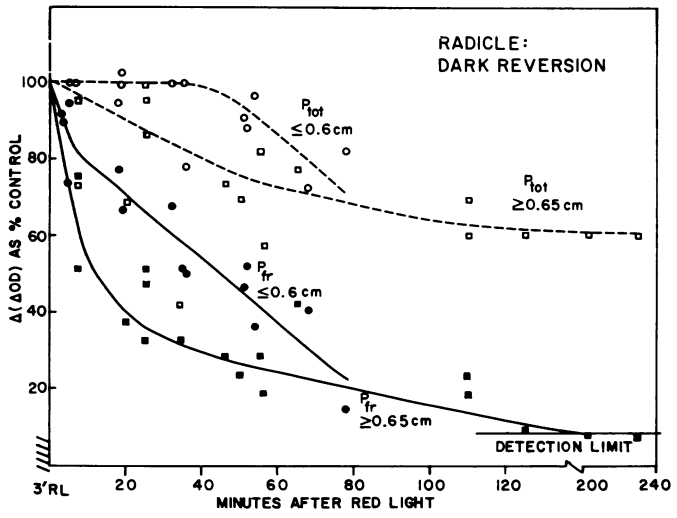

FIG. 1. Dark reversion in different age radicles. After 3 min red light (saturating) samples were examined at various time for Pfr and Ptot. Very young radicles (size average 0.57 cm) show reversion without destruction while older radicles (size average 0.65 cm) do not. Symbols: open circles and open squares are Ptot; closed circles and closed squares are Pfr.

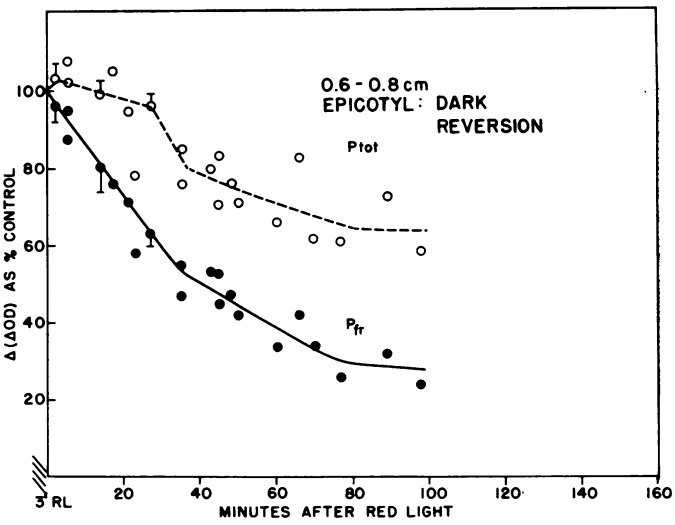

FIG. 2. Dark reversion in the 0.6 to 0.8 cm epicotyl. After 3 min red light (saturating) samples were examined for Pfr and Ptot. Bracket is the standard error of the mean. Points with a bracket represent six samples while all other points represent single samples. Symbols: open circles are Ptot; closed circles are Pfr.

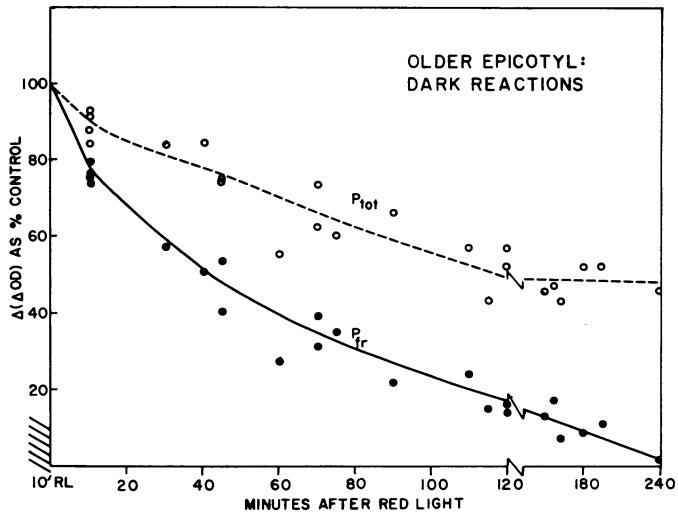

FIG. 3. Phytochrome dark reactions in epicotyls greater than 50 hr old with an average length of 0.60 to 1.10 cm. Symbols: open circles with dashed line are Ptot; closed circles with solid line are Pfr.

started immediately following the red light treatment, 2–4 mm sections of tissue excised just below the apical bud and above the first scale leaf showed rapid reversion of Pfr for at least 20 min in the absence of any destruction. The reversion observed was the most rapid found in any tissue examined in the present study (30% in 10 min).

Other Evidence for Reversion. In both young radicles and young epicotyls in which reversion without destruction could be observed during 30 min immediately following red light treatment, destruction was ultimately observed. Even after this destruction had started, however, a second red light exposure showed the presence of far more Pr than could be accounted for by unmasking of Pr present from the start, or by preferential loss of Pfr, as was the case with maize (7).

If tissues are exposed to continuous red light, any Pfr reversion should be rapidly cancelled as the illumination maintains a photostationary equilibrium between Pr and Pfr. With young

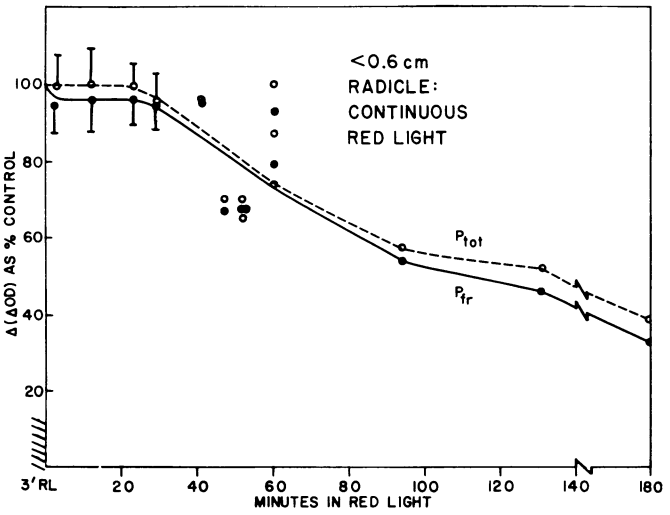

FIG. 4. Phytochrome disappearance under continuous red light in the young radicle. Bracket represents the standard error of the mean. Symbols: points with bracket represent six samples whereas other points are single samples; open circles: Ptot; closed circles: Pfr.

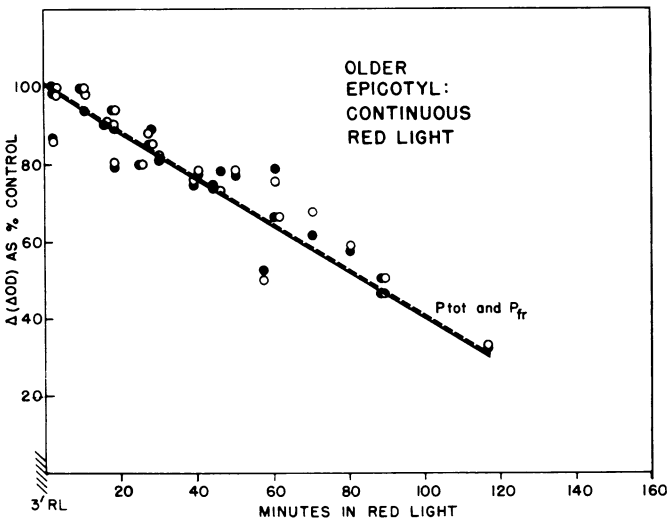

FIG. 5. Phytochrome changes in older epicotyls under continuous red light. Symbols: open circles: Ptot; closed circles: Pfr.

radicles (Fig. 4), examination of tissues at various times during continuous irradiation reveals no change either in total phytochrome or Pfr during the same initial 30-min period. The absence of any such change under these cycling conditions further emphasizes that what was observed during the first 30 min after a single red light treatment must have been reversion. Once again, after 30 min, destruction is evident. Identical results were obtained with young epicotyls.

Even when destruction and reversion may be going on simultaneously, as with older epicotyls, one can obtain strong indirect evidence for reversion. Figure 5 illustrates the time course for loss of Pfr and total phytochrome in older epicotyls. As expected under continuous red illumination, the two proceed in parallel and essentially linearly. These data should be compared with those in Figure 3, illustrating the dark reactions of the same tissue after a single red light exposure. Under cycling conditions (Fig. 5), the rate of loss of Pfr is significantly less than the rate in the dark (Fig. 3) despite the fact that total phytochrome is declining more rapidly in the cycled preparation. Furthermore, the kinetics of Pfr loss in the dark are nonlinear, showing an initial rapid phase which is missing in the cycled sample. Dark reversion during the first 30 min (Fig. 3) provides the most reasonable explanation for the difference. This explanation is strongly supported by experiments with inhibitors of destruction of Pfr in pea tissue (13). Hopkins and Hillman (17) based a similar conclusion on kinetic considerations of dark reactions of Pfr in several dicotyledonous seedlings.

In the present study, sodium azide (2 mM) allowed reversion in 0.5 cm radicles to proceed with little or no destruction. In the cold (samples kept on ice), though the over-all rate of reversion was expectedly slow, it showed an initial rapid phase for about the first 2 hr, followed by a slower phase, thus resembling *in vivo* reversion previously described for cauliflower florets (8).

DISCUSSION

With the exception of *Amaranthus caudatus* (18), evidence for reversion of Pfr to Pr has been obtained in all dicotyledonous tissues examined. In some cases, it is quite rapid, as in the *Cynara* receptacle (14). In the growing tissues of the young etiolated pea seedling where reversion may be observed in the absence of destruction, one could perhaps better assess the role of reversion in physiological responses than in systems in which reversion is difficult to separate quantitatively from simultaneous destruction.

Even when the two dark reactions occur simultaneously, as with older pea tissue, comparison of the rate of loss of Pfr following a single saturating red treatment with that during continuous red irradiation may provide data on the kinetics of reversion. However, comparison of Pfr loss, in segments of stems of 7-day-old Alaska peas grown in the dark, under continuous red irradiation (9) with Pfr loss in similar tissue irradiated and then placed in the dark (11) shows only a very small difference which might be attributed to reversion. There is a further complication in that phytochrome which is being cycled by continuous irradiation has at photostationary state a substantial amount of long lived intermediates present (5). There is at present no evidence on the relative labilities of Pfr *versus* these intermediates, but unless they are the same or at least quite similar, comparison of dark loss of Pfr with loss during continuous irradiation will not give any meaningful quantitative data. Comparison of loss of total phytochrome in Figure 3 (dark reaction) and Figure 5 (continuous illumination) indeed suggests that the intermediates may be more labile, since the amount of loss of total phytochrome is significantly greater in tissue in which the phytochrome is being cycled.

There are two changes in the two dark reactions of Pfr during development which should be noted. First, the initial rate of reversion decreases as juvenile tissue ages. Second, juvenile tissue invariably exhibited a substantial lag between the end of irradiation and the onset of Pfr destruction, something not observed in more mature tissue. This lag is similar to that seen in corn mesocotyl tissues where reversion is absent (8). It may be that destruction in tissues showing the lag requires some component which itself is regulated by Pfr, a component which gradually accumulates in the dark as the tissues age. A substance which rapidly inactivates Pfr *in vitro* has indeed been obtained in crude extracts of pea seedlings (12), but nothing is known of its concentration in the tissue at various developmental stages, nor is it known whether or not it plays a role in Pfr destruction *in vivo*.

With isolated phytochrome, the fastest reversion thus far reported was in partially purified material from parsnip leaves (19). Partially purified pea phytochrome reverted more slowly, and the rate changed with time (as was the case *in vivo* in the chilled young radicle). Anderson *et al.* (1) have recently shown that reversion in purified oat phytochrome is strongly dependent upon temperature and pH, and have not shown that at acidic pH, the rate

shows some temperature compensation. They suggest involvement of dark reversion in physiological reactions regulated both by light and temperature. It is clear that relating *in vitro* studies of phytochrome reversion to physiological responses requires at minimum a system in which reversion can be studied quantitatively *in vivo*. The juvenile tissues of etiolated pea seedlings could provide such a system.

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