Photochemical and Nonphotochemical Reactions of Phytochrome in vivo[1]

Lee H. Pratt[2] and Winslow R. Briggs
Department of Biological Sciences, Stanford University, Stanford, California

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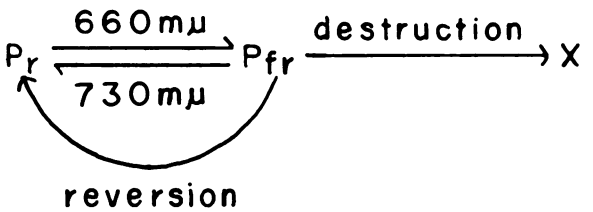
Summary. The nonphotochemical reactions of phytochrome in the coleoptiles of dark-grown corn seedlings were studied at 3 temperatures: 14°, 24°, and 34°. The data obtained show that the destruction of P_{fr} is the only measurable reaction occurring; reversion of P_{fr} to P_r was not found. The Q_{10}'s (2.7 and 3.5) and zero order kinetics found for the destruction reaction are consistent with the hypothesis that the reaction is enzyme-mediated.

In vivo action spectra for phytochrome transformation in the coleoptiles of dark-grown corn seedlings were obtained which agree qualitatively with those obtained by other workers for phytochrome-mediated physiological responses and in vitro action spectra. In vivo conversion of phytochrome by blue light, as determined from spectrophotometric measurements of phytochrome itself, is reported. Action peaks for P_r were found at 667 mμ and in the blue in the region of 400 mμ, with a broad shoulder from 590 mμ to 640 mμ. Action peaks for P_{fr} were found at 725 mμ and in the blue in the region of 400 mμ, with a minor peak at 670 mμ, and a broad shoulder from 590 mμ to 640 mμ. The ratio of the quantum efficiencies of P_r at 667 mμ and P_{fr} at 725 mμ (ϕ_{r667}/ϕ_{fr725}) was estimated to be 1.0.

Many aspects of plant development are now known to be controlled by the photoreversible pigment phytochrome. Phytochrome has been found to take part in both photochemical and nonphotochemical reactions,



where P_r represents the red absorbing form of phytochrome, P_{fr} represents the far-red absorbing form of phytochrome, and X represents the fate of P_{fr} following the destruction reaction. Action spectra have been obtained for phytochrome-mediated responses, such as flowering and seed germination, in many systems (2). Recently, Butler et al. (8) have obtained action spectra for phytochrome transformation in purified extracts of dark-grown oat seedlings. Nonphotochemical reversion of phytochrome was first suggested by the work of Borthwick et al. (5) on lettuce seed germination. Both reversion and destruction have now been measured directly in a variety of plant tissues (9, 10, 13, 14, 15). One does not necessarily find both reactions in any given tissue. For instance, Butler and his associates (9, 10) found reversion but not destruction of phytochrome in cauliflower, and destruction but not reversion in dark-grown corn seedlings. Similarly Hillman (14) has described reversion apparently unaccompanied by destruction in *Pastinaca sativa* (parsnip) and *Cynara scolymus* (artichoke) tissues. Reversion denotes here nonphotochemical transformation of P_{fr} to P_r, while destruction denotes the loss of detectable phytochrome reversibility via P_{fr}.

The present paper examines both the photochemical and nonphotochemical reactions indicated above. Action spectra for phytochrome transformation in vivo are presented and compared to in vitro, as well as physiological, action spectra obtained by other workers.

Materials and Methods

Corn seedlings (*Zea mays* L., cultivar, Barbecue Hybrid) were grown in the dark after the method described by Briggs (6), except that they received no red light prior to experimental use. The procedure consisted of surface-sterilizing the seeds, and then soaking them in running deionized water for 10 to 14 hours in the dark. The seeds were then germinated in flat bowls on agar and, after germination, placed individually into vials filled with 1 % agar for

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[2] Present address: Botany Department, Oregon State University, Corvallis, Oregon.

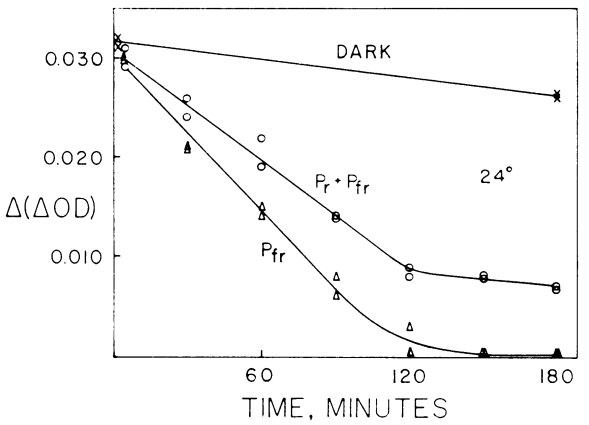
support. They were grown at 24 ± 0.5° and 92 ± 2% humidity. The Barbecue Hybrid variety was used for 3 reasons: 1) the dark-grown coleoptiles have a relatively high concentration of phytochrome; 2) the primary leaf is approximately 5 mm shorter than the coleoptile when the seedlings are about 84 hours old, making it possible to obtain coleoptile samples free of primary leaf tissue; and 3) the coleoptiles are virtually devoid of protochlorophyll.

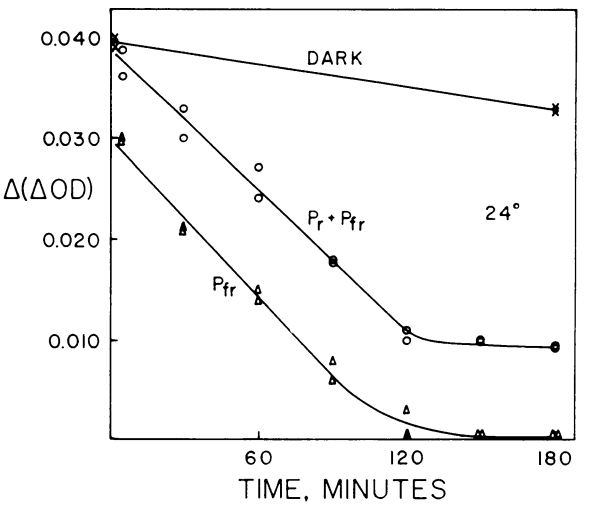
A description of the instrument and necessary techniques used for the measurement of phytochrome is given by Butler et al. (10). A Ratiospect (model R-2, Agricultural Specialty Company, Beltsville, Maryland) functionally identical to the double-beam difference spectrophotometer described by Butler et al. was used for the work reported below. All phytochrome measurements were made with the sample in an aluminum cell maintained at ice temperature to prevent the thermochemical destruction of P_{fr}. The cell has a cross-sectional area of 1 cm² and can hold samples up to 8 mm in depth.

Nonphotochemical Reactions. In vivo nonphotochemical reactions of phytochrome were studied in dark-grown seedlings, approximately 85 hours after the beginning of imbibition. At this stage, the seedlings are about 60 mm in length. From 180 to 260 plants of comparable size were selected at the beginning of each experiment so that there would be enough for 20 plants per sample, each experiment using from 9 to 13 samples. Each sample consisted of approximately the apical 15 mm of the coleoptiles, freed from primary leaf, and weighed 0.41 ± 0.02 g. All handling of the material was done in a dark growth room under dim green light at 24 ± 0.5° and 92 ± 2% humidity. The green light used was ineffective in photoconverting phytochrome.

At the beginning of each experiment, all plants, except for 2 groups to be used as dark controls, were illuminated for from 5 to 10 minutes with red light (sufficient to drive the pigment as far as possible to the red-absorbing form) at the desired temperature. The plants were then placed in the dark and kept at the specified temperature (14°, 24°, or 34°) until examined spectrophotometrically. Controls received no red light treatment, but were kept at the experimental temperature for the time indicated. The red light source in this series of experiments consisted of two circular fluorescent tubes (daylight, 32 and 40 w) placed concentrically with the light filtered through 3 layers of red cellulose acetate. The plants were placed approximately 25 cm from the source.

Action Spectra. In order to get comparable results from 1 sample to the next, it was necessary to prepare the samples in a standard manner. Each sample consisted of the top 1 to 2 mm of 150 coleoptiles and weighed 0.16 ± 0.005 g. The tips were previously found to have a higher concentration of phytochrome than the remainder of the coleoptile (7). The samples were about 2 mm thick. A sample any thinner than 2 mm could not be used because it would have holes through which light would pass





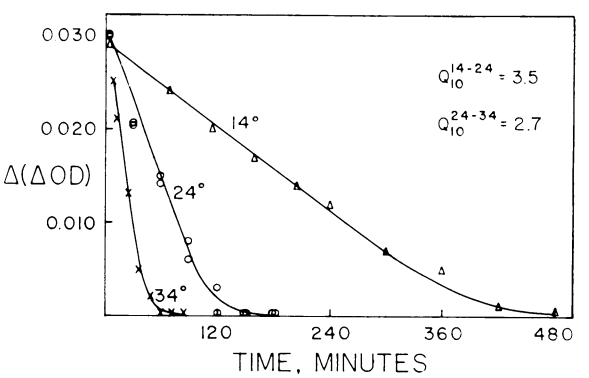


FIG. 1 (*top*). Phytochrome changes in the dark at 24° in coleoptiles of 85-hour-old, dark-grown corn seedlings after a 5-minute exposure to red light [see (11)]. ×, dark controls, total phytochrome; ○, red treated, total phytochrome; △, red treated, P_{fr} only.

FIG. 2 (*center*). Phytochrome changes in the dark at 24° in coleoptiles of 85-hour-old, dark-grown corn seedlings after a 5-minute exposure to red light, corrected for the 80:20 ratio of $P_{fr}:P_r$ produced by red light.

directly, thereby decreasing the resolution of the ratiospect. The samples were prepared under dim green light and immediately placed into the aluminum measuring cell which was then inserted into an ice bucket. Thus subsequent light-induced absorbance changes occurred at temperatures close to 0°.

Various light sources were used for different parts of the spectrum. The blue and long ultraviolet wavelengths (365, 405, 436, 546, and 557 mμ) were isolated from a low pressure mercury arc with a Bausch and Lomb grating monochromator. Light of 467 mμ was isolated from the spectrum of a 75-w high pressure xenon arc. A 500-w projector and interference filters were used to isolate wavelengths at 650, 667, 682, 725, and 743 mμ. The remainder of the wavelengths tested were obtained from a Bausch and Lomb grating monochromator with a tungsten light source.

An 8-junction thermopile (Eppley No. 3979), calibrated against a standard lamp from the National Bureau of Standards, was used for all measurements of light intensity. The value obtained from the thermopile, in microvolts measured with a Hewlitt-Packard microvoltmeter, was then converted to Einsteins $\cdot cm^{-2} \cdot sec^{-1}$ for each wavelength.

Results

Nonphotochemical Reactions. By illuminating dark-grown seedlings with red light for a short period of time and returning them to the dark, one may follow changes in phytochrome by examining samples of the seedlings at different times after returning them to the dark. Such an experiment done at 24° shows loss of total phytochrome reversibility and an apparent reversion of P_{fr} to P_r. However, as Butler et al. (11) have shown for phytochrome extracts from dark-grown oat seedlings, and DeLint and Spruit (13) have suggested for corn mesocotyls, red light sets up a photostationary equilibrium with only 80 % of the phytochrome in the far-red absorbing form. It is assumed here that the same equilibrium is also obtained in vivo in corn coleoptiles. The raw data at 24° are presented in the earlier manner of Butler et al. (10) (fig 1). Figure 2 shows the data replotted on the basis of total phytochrome. Since red light sets up an 80:20 equilibrium of $P_{fr}:P_r$, the total amount of reversible phytochrome present is the measured $\Delta(\Delta OD)/0.80$. When all the $\Delta(\Delta OD)$ readings are adjusted on this basis, one obtains the curves shown in figure 2 for phytochrome concentration in the dark controls (dark) and red light-treated plants ($P_r + P_{fr}$). Since far-red light sets up a photostationary equilibrium with approximately 99 % of the phytochrome in the red absorbing form (10), the initial $\Delta(\Delta OD)$ shift upon illumination with far-red light gives an accurate measure of the actual amount of P_{fr} present, within the limits of sensitivity of the ratiospect.

For figures 1 and 2, a separate sample was used to obtain the data for each point on the $P_{fr} + P_r$ and P_{fr} curves. Two separate experiments were done at 24°, with the data from both shown in both figures. One experiment each was also done at 14° and 34°.

The lack of measurable reversion of P_{fr} is evident when one notes that the $P_r + P_{fr}$ and P_{fr} curves in figure 2 are parallel. The vertical differences between the 2 curves is a measure of the amount of P_r present, this difference being constant. Since the concentration of P_r does not change measurably, no observable P_r is being formed by the reversion of P_{fr}. Identical results were obtained at 14° and 34°.

The destruction reaction (P_{fr} curve in fig 1) follows zero order kinetics. Butler et al. (10) observed that only about 10 % of the phytochrome in a dark-grown corn seedling need be present as P_{fr} to saturate the destruction reaction. The shape of the curves at the 3 temperatures studied (see fig 3) also indicates that the reaction causing loss of reversibility is saturated with P_{fr} until only about 10 % of the P_{fr} initially present is left, at which point the curve becomes nonlinear.

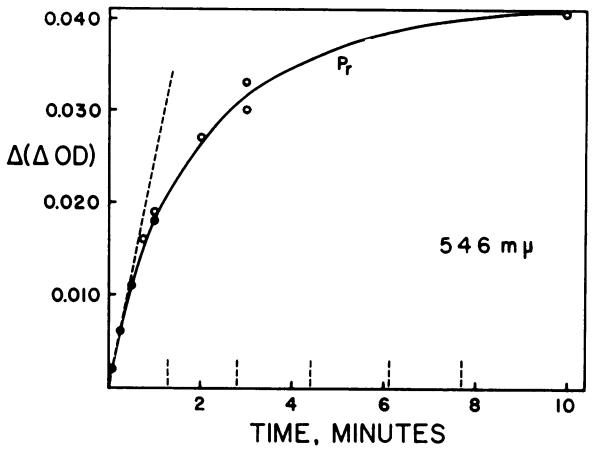
Figure 3 shows the loss of P_{fr} plotted against a common time axis for all 3 temperatures. It is clear from this graph that the destruction reaction has a Q_{10} of about 3.

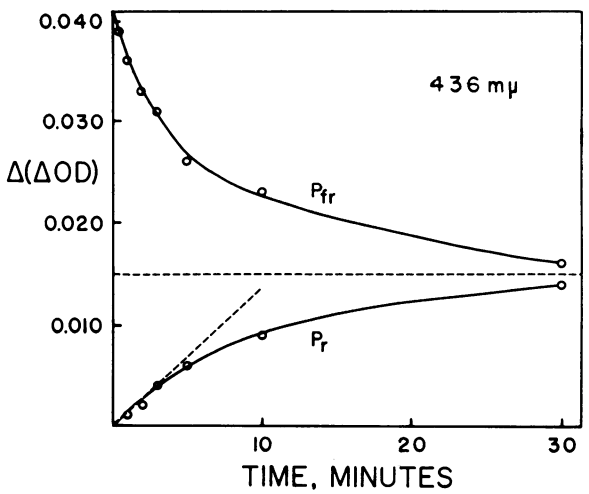
Action Spectra. Three methods of obtaining the values for the action spectra for phytochrome transformation are used. These methods are outlined below. In measuring the rate of transformation of P_r to P_{fr}, the photochrome was always initially present as P_r only, while in measuring the rate of transformation of P_{fr} to P_r, the phytochrome was always initially present in an 80:20 ratio of $P_{fr}:P_r$. The initial condition of phytochrome is obtained by irradiation with light at 730 mμ in the former instance, and at 663 mμ in the latter case.

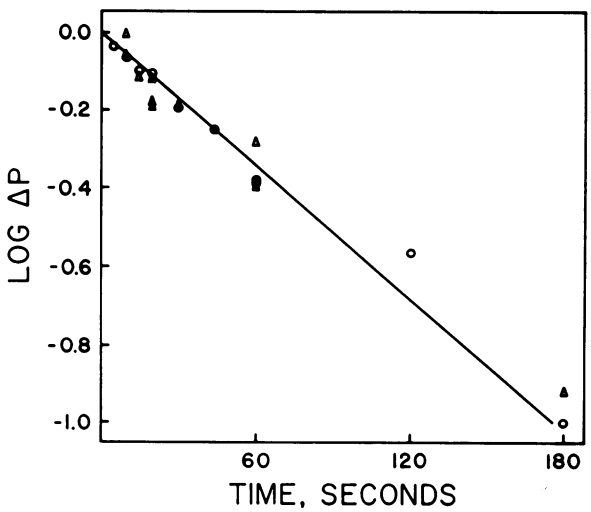
Figure 4 shows a typical curve obtained by measuring the change of P_r to P_{fr} under the influence of a light source (546 mμ) which establishes the same photostationary equilibrium as the actinic red light in the ratiospect. In such a case, measurable transformation is seen only from P_r to P_{fr} and not P_{fr} to P_r, although the latter must occur in order that the 80 : 20 equilibrium be established. The method of determining the initial rate of the reaction, which is used in determining the action spectrum for P_r, is indicated by the dashed construction line in figure 4. The dashed line is tangent to the initial portion of the curve. The initial rate is expressed as the change in ($\Delta OD \cdot sec^{-1}$/total phytochrome) × 100. The total phytochrome is used since this represents the

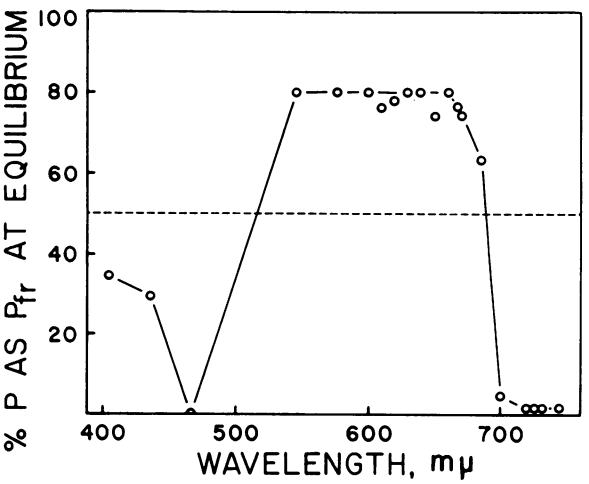
×, dark controls, total phyotochrome; ○, red treated, total phytochrome; △, red treated, P_{fr} only.

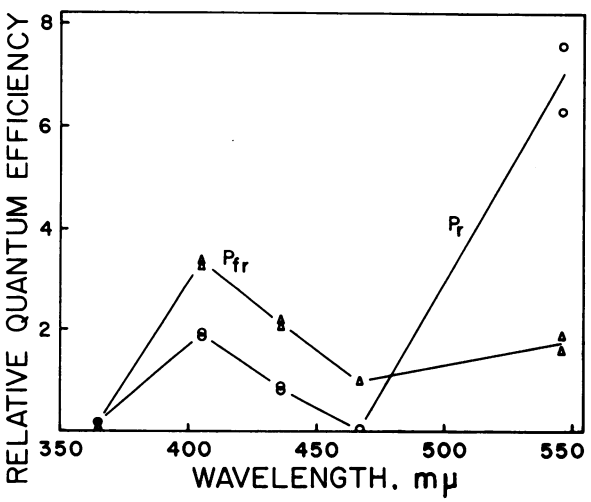
FIG. 3 (*bottom*). Changes in P_{fr} concentration with time at various temperatures, plotted on a common time axis, indicating Q_{10}'s for the destruction reaction. △, 14°; ○, 24°; ×, 34°.











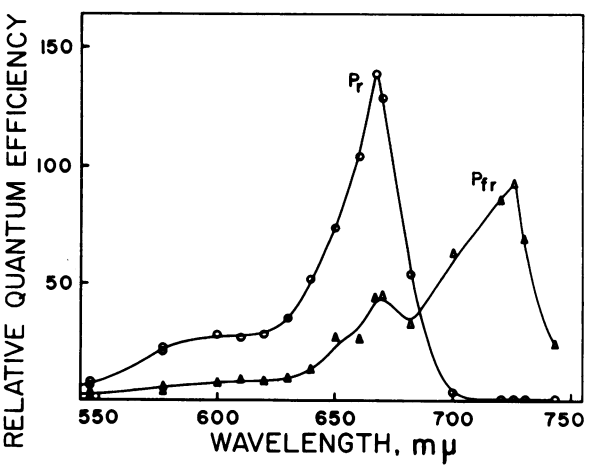


FIG. 4 (*upper left*). The photoconversion of P_r to P_{fr} with time by light of 546 mμ in the coleoptiles of dark-grown seedlings. The initial rate of the reaction is indicated by the dashed line tangent to the initial portion of the curve. The dashed lines parallel to the ordinate indicate the half-times for the reaction.

FIG. 5 (*upper right*). The photoconversion of P_r to P_{fr} and of P_{fr} to P_r with time by light of 436 mμ in the coleoptiles of dark-grown corn seedlings. The initial rate of the reaction of P_r to P_{fr} is indicated by the dashed con-

portion of phytochrome initially present as P_r, where total phytochrome = $\Delta(\Delta OD)_{measured}/0.8$. The initial rate is then converted to percent transformation per Einstein which in turn is plotted against wavelength (fig 8, 9) as relative quantum efficiency. Energy is expressed as Einsteins·cm^{-2}·sec^{-1}.

A second method is required when the wavelength being studied sets up other than an 80:20 ratio of P_{fr}:P_r. Figure 5 (436 mμ) illustrates this situation. The initial rate for the conversion of P_r to P_{fr} is again shown by the dashed line, and is calculated in the same manner. The photostationary equilibrium is indicated by the horizontal dashed line. Since essentially all of the phytochrome is present as P_r at the beginning of the light exposure to 436 mμ, after having been irradiated with far-red light, we may consider the first 10 % of the reaction to be free of the influence of the competing back reaction, i.e., P_{fr} to P_r. However, in determining the rate of the reaction P_{fr} to P_r, one must note that the measured initial rate is not comparable to the one for the reaction P_r to P_{fr}, since 20 % of the phytochrome is already in the P_r form and hence competing with the reaction of P_{fr} to P_r. To overcome this obstacle we may use the relationship $k_{fr} = k_r P_{r^z}/P_{fr^z}$, where k_r is the rate constant for P_r, P_{r^z}/P_{fr^z} is the ratio of the concentrations of P_r and P_{fr} at photostationary equilibrium, and k_{fr} is the rate constant for P_{fr}. The initial rates of the 2 competing reactions are in the same ratio as k_{fr}: k_r, and therefore the initial rate of change of P_{fr} may be determined from the measured rate of change of P_r at the same wavelength and the photostationary equilibrium established by that wavelength. These computed values make up the action spectrum of P_{fr} in figures 8 and 9, from 365 mμ through 682 mμ.

The third method is used for wavelengths greater than 682 mμ, where it is possible to measure the rate of change of P_{fr} with time. This reaction is unobstructed by the reverse reaction of P_r to P_{fr}, which at these wavelengths is negligible (ca. 1 % of the P_{fr} value). Even though 20 % of the phytochrome is present as P_r initially, there is almost no reaction of P_r to P_{fr} to compete with the transformation of P_{fr} to P_r, so that initial rates for the photoconversion of P_{fr} may be computed just as for the photoconversion of P_r. However, the substrate (P_{fr}) present at the beginning of the reaction is the measured $\Delta(\Delta OD)$ rather than the total $\Delta(\Delta OD)$ since only 80 % of the phytochrome present is in the P_{fr} form at photostationary equilibrium with red light. Hence, the initial rate (as % transformation·sec^{-1}) is the change in $(\Delta OD \cdot sec^{-1}/\Delta(\Delta OD)_{measured}) \times 100$.

The relative quantum efficiencies at each wavelength for the photoconversion of P_r and P_{fr} are determined from a single sample. In many cases, the same sample is used to determine relative quantum efficiencies for 2 or more wavelengths.

The kinetics for the photochemical reactions are first order (8). The dashed lines along the abscissa of figure 4 indicate the half-times for the reaction. The values for the half-time vary from 1.3 to 1.7 minutes, increasing slightly as the reaction proceeds. The slight increase in half-time is a result of the increasing influence of the reverse photoreaction ($P_{fr} \rightarrow P_r$) on the one being studied ($P_r \rightarrow P_{fr}$).

Figure 6 presents further evidence showing that both photochemical reactions ($P_{fr} \rightarrow P_r$ and $P_r \rightarrow P_{fr}$) are first order. Log ΔP is plotted against time for the 2 photoreactions at 682 mμ, where

$$\Delta P = \frac{(P_r) - (P_{r^z})_{682}}{(P_{ro}) - (P_{r^z})_{682}} \text{ (open circles) or}$$

$$\Delta P = \frac{(P_{fr}) - (P_{fr^z})_{682}}{(P_{fro}) - (P_{fr^z})_{682}} \text{ (triangles);}$$

and (P_r), or (P_{fr}), is the concentration of P_r, or P_{fr}, at the time indicated; $(P_{r^z})_{682}$, or $(P_{fr^z})_{682}$, is the concentration of P_r, or P_{fr}, at the photostationary equilibrium established by 682 mμ light; and (P_{ro}), or (P_{fro}), is the concentration of P_r, or P_{fr}, at time zero (P_{ro} = total phytochrome; P_{fro} = measured phytochrome). A derivation and discussion of this relationship is given by Butler et al. (8). If the 2 re-

struction line tangent to the initial portion of the lower curve. The dashed line parallel to the abscissa indicates the photostationary equilibrium established by light of 436 mμ. Lower curve, the reaction $P_r \rightarrow P_{fr}$, initially irradiated with far-red light; upper curve, the reaction $P_{fr} \rightarrow P_r$, initially irradiated with red light.

FIG. 6 (*center left*). Plot of log ΔP against time for photoconversion of P_r to P_{fr} and to P_r by light of 682 mμ in the coleoptiles of dark-grown corn seedlings. ○, $\Delta P = \frac{(P_r) - (P_{r^z})_{682}}{(P_{ro}) - (P_{r^z})_{682}}$, sample initially irradiated with far-red light; △, $\Delta P = \frac{(P_{fr}) - (P_{fr^z})_{682}}{(P_{fro}) - (P_{fr^z})_{682}}$, sample initially irradiated with red light.

FIG. 7 (*center right*). Plot of percent phytochrome present as P_{fr} at photostationary equilibrium against wavelength.

FIG. 8 (*bottom left*). Action spectra of the photoconversion of P_r and P_{fr} in the blue. ○, Photoconversion of P_r to P_{fr}, sample initially irradiated with far-red light; △, photoconversion of P_{fr} to P_r, sample initially irradiated with red light. Units of relative quantum efficiency are (percent transformation/Einstein) (10^{-8}).

FIG. 9 (*bottom right*). Action spectra of the photoconversion of P_r and P_{fr} in the red region of the spectrum. ○, Photoconversion of P_r to P_{fr}, sample initially irradiated with far-red light; △, photoconversion of P_{fr} to P_r, sample initially irradiated with red light. Units of relative quantum efficiency are (percent transformation/Einstein) (10^{-8}).

actions being studied are first order, the data of figure 6 should yield a straight line, which is the best straight line through the data both for the photoconversion of P_r to P_{fr} (open circles) and P_{fr} to P_r (triangles). Inspection of figure 6 will show that such a straight line was obtained.

The data obtained for the relative quantum efficiencies of both reactions ($P_r \rightarrow P_{fr}$ and $P_{fr} \rightarrow P_r$) by the various methods described above are listed in table I, along with the photostationary equilibrium values of P_r and P_{fr} and incident energies used at the different wavelengths studied. Figure 7 shows the relative amounts of P_r and P_{fr} present at photostationary equilibrium, plotted against wavelength. A prominent feature of the graph is that none of the wavelengths used could convert more than 80 % of the phytochrome to P_{fr}.

The action spectrum for P_r (fig 8, 9) has a peak at 667 mμ, with a pronounced shoulder from about 580 mμ to 620 mμ. The action spectrum for P_{fr} (fig 8, 9) has a major peak at approximately 725 mμ, with a minor one at about 670 mμ. The latter peak was not as prominent in an action spectrum for transformation of P_{fr} extracted from oat seedlings (8). Both action spectra show peaks near 400 mμ. Note that the relative quantum efficiency scales are not the same for the 2 figures.

The action spectra for photoconversion of P_r and P_{fr} show that the relative effectiveness of red light is of the order of 100 times that of blue for P_r, and the ratio of the effectiveness of far-red to blue light for P_{fr} is about 25. These ratios are of the same order of magnitude as those found by investigators at the Agricultural Research Station in Beltsville, Maryland, in much of their earlier physiological work (3, 16, 17) but are far greater than the ratios obtained in vitro (8).

Discussion

The in vivo action spectra for phytochrome transformation in corn (fig 8, 9) agree quite well with those obtained for physiological responses, including flowering (2), change in phototropic sensitivity (12), and seed germination (4, 19). Close agreement is also obtained between the in vivo action spectra in figures 8 and 9 and the in vitro action spectra of Butler et al. (8). The in vitro action spectra of Butler et al. lack, however, the broad shoulder between 580 mμ and 630 mμ in figure 10, but this lack may be due to differences in screening and scattering by the 2 widely different systems, or to differences between the phytochromes of corn and oats.

The initial rate of transformation of either P_{fr} to P_r or P_r to P_{fr} at a given wavelength is a function of 3 variables: 1) the extinction coefficient of phytochrome, 2) the quantum efficiency, and 3) screening

Table I. *Intensity of Light at Each Wavelength, Relative Quantum Efficiencies for Photoconversion for P_r and P_{fr}, and Relative Amounts of P_r and P_{fr} Present at Photostationary Equilibrium*

Wavelength mμ	Intensity* (× 10^{10})	Relative quantum efficiency** P_r (× 10^{-8})	P_{fr} (× 10^{-8})	P_{r^x}***	P_{fr^x}***
365	5.59	<0.25	<0.25	...	...
405	11.10	1.88	3.34	64	36
405	17.30	1.93	3.43		
436	5.26	0.85	2.1	71	29
436	18.60	0.89	2.2		
467	4.36	0.0	0.97	100	0
546	10.55	7.6	1.9	20	80
546	9.45	6.3	1.6		
577	9.90	21	5	20	80
577	9.76	22	5		
600	0.44	28	7	20	80
610	0.55	27	9	25	75
620	0.52	28	8	22	78
630	0.58	35	9	20	80
640	0.56	52	13	20	80
650	1.47	74	27	27	73
660	0.51	104	26	20	80
667	2.02	139	44	24	76
670	0.49	129	45	26	74
682	1.66	54	32	37	63
700	0.61	3	63	95	5
720	0.93	0	86	99	1
725	1.29	0	93	99	1
730	0.94	0	69	99	1
743	5.53	0	24	99	1

* Intensity given as Einsteins•cm^{-2}•sec^{-1}.
** Relative quantum efficiency expressed as percent transformation/Einstein.
*** P_{r^x} (P_{fr^x}) is relative amount of P_r (P_{fr}) at photostationary equilibrium.

by the coleoptile, all at the wavelength being used. Therefore, $r = \epsilon\phi\beta$, where r is the initial rate, expressed as percent transformation per second, ϵ is the extinction coefficient, ϕ is the quantum efficiency, expressed as percent transformation per Einstein, and β represents screening by the coleoptile tissue. Assuming that screening is the same at both 667 mμ and 725 mμ, and that the ratio of ϵ_{r667} to ϵ_{fr725} is 1.55 (8), we may compute the ratio of ϕ_{r667} to ϕ_{fr725}:

$$\frac{\phi_{r667}}{\phi_{fr725}} = \frac{r_{r667}}{r_{fr725}} \times \frac{\epsilon_{fr725}}{\epsilon_{r667}} = \frac{139}{93 \times 1.55} = 1.0.$$

This ratio is somewhat less than the 1.5 obtained by Butler et al. (8), but it must be noted that a probable differential in screening by the coleoptiles has not been accounted for.

The distinct peak in the action spectrum for transformation of P_{fr} to P_r at about 670 mμ indicates that P_{fr} possesses a real absorbancy peak at this wavelength. Thus the peak seen in the absorption spectrum of P_{fr} at this wavelength (18) must have 2 components: the 20 % P_r found at photostationary equilibrium with red light and the secondary peak of P_{fr}.

Evidence for the reversion of P_{fr} to P_r is known from physiological studies (5), in vivo measurements (9, 10, 14) and in vitro studies (11). The physiological evidence is based on work with germination of lettuce seeds. The in vivo measurements showed the reversion of P_{fr} to P_r without loss of reversibility in cauliflower, parsnip, and artichoke. It is important to note that the kinetics for reversion in cauliflower (where sufficient data are available for comparison) are strikingly different from those for dark destruction of P_{fr} described in the present paper. Reversion in cauliflower does not follow zero order kinetics as does dark destruction of P_{fr} in corn. The in vitro studies were done with phytochrome extracts which showed no reversion unless they were aged or the phytochrome was partly denatured (spectroscopically altered). Butler et al. (11) could show no dark reversion in fresh, purified solutions of phytochrome from oats, but could after aging or mild denaturation. The work described in the present paper shows that there is no measurable reversion of P_{fr} to P_r in corn coleoptiles.

The zero order kinetics obtained for the destruction of P_{fr} suggest that some substance other than P_{fr} is limiting the rate of the reaction. Such results are consistent with the hypothesis that the destruction of P_{fr} is enzyme-mediated, with an enzyme, the rate-limiting substance. The destruction of P_{fr} has been shown to be oxygen-dependent by Butler and Lane (9). Butler and Lane also found that the destruction reaction is inhibited in a manner paralleling inhibition of respiration by lowering O_2 tension or adding respiratory poisons (CO, KCN, and sodium azide). In addition, the Q_{10} for the reaction (fig 3) is of an order of magnitude (ca. 3) consistent with enzyme mediation. The evidence described above, then, supports the hypothesis that the destruction of P_{fr} is enzyme-mediated.

The kinetics of phytochrome destruction shown here are not necessarily typical of all plant materials. For instance, Hopkins and Hillman (15) have shown that disappearance of P_{fr} in hypocotyls of dark-grown *Raphanus, Phaseolus,* and *Glycine* has an initial rapid phase lasting about an hour, followed by a slow phase which reaches completion after an additional 3 or 4 hours. Their data are complicated, however, by the fact that the equilibrium percentages of P_r and P_{fr} following saturating red light treatments are not known for these 3 plants, and therefore plotting total pigment instead of just that measured (cf. fig 1, 2) is not possible. It is possible as they suggest that some reversion is occurring in all 3 plants, further complicating kinetic analysis of P_{fr} destruction.

The action spectra for phytochrome transformation (fig 8, 9) and the equilibrium ratios at photostationary equilibrium for different wavelengths (fig 7) indicate the need for considering the possible role of phytochrome in physiological responses that do not seem to fit the classic phytochrome pattern. Bertsch (1) has demonstrated the reversal of blue light effects in *Pisum* by far-red light, showing that blue light in this system may exert its effect through phytochrome. Examination of figure 8, as well as action spectra obtained in vitro by Butler et al. (8) and obtained for physiological responses (2), corroborate the finding that blue light effects may be phytochrome-mediated. Action spectra of all 3 types (in vivo conversion, in vitro conversion, and physiological response) show activity in the blue region. The absorption spectrum of purified phytochrome (18), showing prominent peaks in the region near 400 mμ both for P_r and P_{fr}, of course, constitutes the most direct evidence that one should expect blue light to mediate phytochrome-regulated physiological responses.

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