Physicochemical Differences between the Red- and the Far-Red-Absorbing Forms of Phytochrome†

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ABSTRACT: We investigated some of the chemical and physical differences between the red-absorbing (Pr) and far-red-absorbing (Pfr) forms of immunoaffinity-purified, undegraded oat (*Avena sativa* L., cv. Garry) phytochrome. Both Pr and Pfr had identical surface charges as measured by isoelectric focusing and identical secondary structure as judged from their circular dichroic spectra. Modification of specific amino acid residues, however, revealed some chemical differences between Pr and Pfr. Compared to Pr, Pfr had one more His and Cys residue per monomer modified immediately. His residues on Pfr were modified more rapidly than were those on Pr, as opposed to Cys modification which, after the initial burst, occurred more rapidly on Pr than on Pfr. Both His- and Cys-modified phytochromes were fully photoreversible. Both Pr and Pfr had the same number of immediately modified carboxyl functions, but those on Pr reacted slightly more rapidly than those on Pfr. Carboxyl-modified phytochrome was denatured by the acid pH used for modification so that its photoreversibility could not be measured. Modification of Tyr on Pr and Pfr resulted in two residues per monomer being modified very rapidly, with those on Pfr reacting even faster than those on Pr. Subsequent slower modification of remaining Tyr residues, however, occurred more rapidly on Pr than on Pfr. Phytochrome photoreversibility declined as a linear function of the number of Tyr modified and was lost completely when two Tyr per monomer had been modified. These data are consistent with the hypothesis of a small conformational change in phytochrome upon photoconversion and also fit a conformation–stabilization mechanism in which photoconversion from Pr to Pfr stabilizes phytochrome in one particular conformation.

Phytochrome, a blue-green chromoprotein, is the photoreceptor for many light-mediated developmental responses in plants (Smith, 1975). The chromoprotein, which is a dimer (Hunt & Pratt, 1980) of its 118 000-dalton monomers (Gardner et al., 1971), exists in one of two repeatedly photointerconvertible forms: a physiologically inactive, red-absorbing form (Pr)[1] and a physiologically active, far-red-absorbing form (Pfr). Photoconversion in either direction occurs in the absence of any known activator or cofactor, requiring only induction by light. Photomorphogenic responses are triggered by Pfr but not Pr (Smith, 1975). Therefore, it must be the difference(s) between Pr and Pfr that leads to these responses.

Most comparisons of Pr to Pfr utilized a proteolytically degraded, apparently nonphysiological form of the molecule [cf. Pratt (1979) for review of this work]. Only a few attempts to detect differences between undegraded Pr and Pfr have been reported. No differences were noted by using immunochemical methods (Pratt, 1973; Rice & Briggs, 1973; Cundiff & Pratt, 1975). Tobin & Briggs (1973) noted small differences in ultraviolet absorption while Tobin & Briggs (1973) and Song et al. (1979) saw no differences by circular dichroism in the far-ultraviolet region. Song et al. (1979), however, pointed out that the undegraded phytochrome with which they worked was only about 20% pure. As they noted, the comparison that they made between Pr and Pfr was thus not rigorous. Gardner et al. (1974) reported that Pfr bound more *N*-[^{14}C]ethylmaleimide than did Pr. In addition, Pfr was spectrally more labile to divalent cations, such as Cu^{2+}, Co^{2+}, and Zn^{2+}, than was Pr (Lisansky & Galston, 1974, 1976; Pratt & Cundiff, 1975).

We report here a study on the physicochemical differences between Pr and Pfr using immunoaffinity-purified, undegraded oat phytochrome. Studies include comparative isoelectric focusing, circular dichroic spectroscopy, and amino acid modifications of undenatured Pr and Pfr.

Materials and Methods

Phytochrome. Undegraded oat (*Avena sativa* L., cv. Garry) phytochrome was immunoaffinity purified as previously described (Hunt & Pratt, 1979). Briefly, phytochrome was partially purified from extracts of 5-day-old, dark-grown oat shoots by $CaCl_2$ treatment, brushite chromatography, and ammonium sulfate fractionation. Partially purifed phytochrome was then adsorbed to agarose-immobilized antiphytochrome immunoglobulins and, after nonadsorbed protein was washed away, phytochrome was eluted with 3 M $MgCl_2$, pH adjusted to 7.5 with Tris. Phytochrome was stored in 0.1 M sodium phosphate, pH 7.8, and 1 mM EDTA, at −76 °C at a concentration of 1 to 1.5 mg/mL. Immunoaffinity-purified phytochrome was greater than 98% homogeneous as judged by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and, except for its dependably higher purity, was identical with conventionally purified phytochrome (Hunt & Pratt, 1979, 1980).

Photoreversibility of native and modified phytochrome was determined at 666 vs. 727 nm in a custom-built, dual-wavelength spectrophotometer (Kidd & Pratt, 1973; Pratt & Marmé, 1976). Pr and Pfr were produced by saturating irradiations with 739- and 667-nm light (Balzers B-40 interference filters), respectively.

Isoelectric Focusing. Isoelectric focusing (pH 3–10) was performed at 2 °C in 5 × 100 mm cylindrical polyacrylamide gels as described by Righetti & Drysdale (1971). Focused

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[1] Abbreviations used: Pr, red-absorbing form of phytochrome; Pfr, far-red-absorbing form of phytochrome; Tris, tris(hydroxymethyl)-aminomethane; EDTA, ethylenediaminetetraacetate; CD, circular dichroic.

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gels were stained for protein with Coomassie blue (Otavsky & Drysdale, 1975).

Histidine Modification. Surface His residues were modified by reaction with diethyl pyrocarbonate (Miles, 1977). Phytochrome (36 μg) was diluted to 297 μL with 50 mM potassium phosphate, pH 6.5, and 3 μL of 40 mM diethyl pyrocarbonate in absolute ethanol (10-fold molar excess over total His) was added. Carbethoxylation was followed by the increase in A_{240} over time in an ice-water-cooled cuvette. The number of modified His was calculated from $\Delta\epsilon_{240} = 3200\ M^{-1}\ cm^{-1}$.

Carboxyl Modifications. Surface carboxyl functions were modified by an adaptation of the procedure of Hoare & Koshland (1967). Phytochrome (240 μg) was precipitated with an equal volume of acetone, collected by centrifugation, and dried under a stream of N_2. The pellet was washed three times with 32 μN HCl (pH 4.5) and then dispersed in 32 μN HCl at a concentration of 2 mg/mL. An aliquot of 15 mL was removed and placed in 100 μL of 1 M sodium acetate, pH 4.5, to provide a blank. An equal volume of 0.67 M [^{14}C]glycine ethyl ester (1.5 mCi/mol) also containing 67 mM 1-ethyl-3-[(dimethylamino)propyl]carbodiimide was added to the remaining phytochrome. Aliquots of 15 μL were removed after increasing time periods and placed in 100 μL of 1 M sodium acetate, pH 4.5. The blank received 15 μL of the reactant solution. Bovine serum albumin (0.5 mg) was added as a carrier. Modified phytochrome was subsequently precipitated and washed twice with 3 mL of acetone–HCl (39:1 v/v). Radioactivity was determined in a liquid scintillation counter.

Tyrosine Modification. Surface Tyr residues were modified by reaction with tetranitromethane (Sokolovsky et al., 1966). Phytochrome (36 μg) was diluted to 297 μL with 50 mM Tris, pH 8.0, at room temperature, and 3 μL of 0.25 M tetranitromethane in absolute ethanol (110-fold molar excess over total Tyr) was added. Nitration was followed at room temperature by the increase in A_{428} over time. Some experiments used 36 μg of phytochrome in 300 μL, final volume, of 50 mM Tris, pH 8.0, at 0 °C, and 3 μL of 25 mM tetranitromethane (11-fold molar excess) in order to slow the initial, rapid reaction to a measurable rate. Other experiments used 160 μg of phytochrome in 600 μL of 50 mM Tris, pH 8.0, at 0 °C, and 6 μL of 0.25 M (50-fold molar excess) or 50 mM (10-fold molar excess) tetranitromethane. In the latter two cases, nitration was monitored in an ice-water-cooled cuvette. The number of modified Tyr was calculated using $\Delta\epsilon_{428} = 4100\ M^{-1}\ cm^{-1}$.

Cysteine Modification. Available Cys residues were modified with dithionitrobenzoic acid (Habeeb, 1972). Phytochrome (30 μg) was diluted to 450 μL in 80 mM sodium phosphate, 0.5 mg/mL tetrasodium EDTA, adjusted to pH 8.0 with HCl, in an ice-water-cooled cuvette, and 50 μL of 4 mg/mL dithiobis(nitrobenzoic acid) (75-fold molar excess over total Cys) in 100 mM sodium phosphate, pH 8.0, was added. The number of modified Cys was calculated using $\Delta\epsilon_{412} = 13\,600\ M^{-1}\ cm^{-1}$.

Circular Dichroic Spectra. Circular dichroic (CD) spectra (200–250 nm) were obtained in a 2-mm path-length cell at 26 °C on a Cary 60 spectropolarimeter equipped with a CD attachment. Triplicate scans were made on each phytochrome preparation (43 μg/mL in 0.1 M sodium phosphate, pH 7.8). The CD spectrum of Pr was obtained first. Phytochrome was then photoconverted to Pfr without moving the cuvette, and the CD spectrum of Pfr was obtained. Ellipticities in millidegrees, θ, were converted to mean residue ellipticities, $[\theta]_{mwr}$, in deg·cm^2/dmol, using an average residue molecular weight of 110.3 (Hunt & Pratt, 1980). Under these conditions, $[\theta]_{mwr} = 1282.6\theta$.

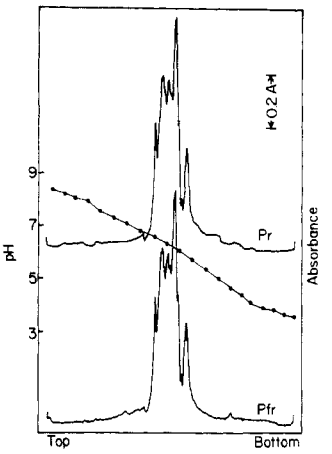

FIGURE 1: Absorbance scans of polyacrylamide gels after isoelectric focusing of 50 μg of immunoaffinity-purified phytochrome focused as Pr or as Pfr and subsequent staining for protein. The pH profile was determined using water-eluted, 0.5-cm slices from a replicate gel.

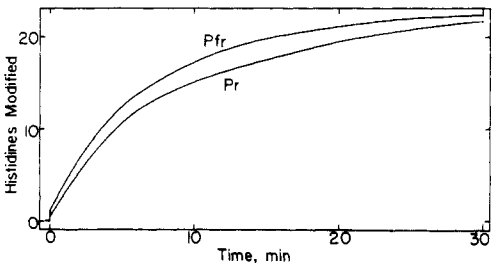

FIGURE 2: Number of His residues per monomer modified as a function of time in the presence of a 10-fold molar excess of diethyl pyrocarbonate over total His at pH 6.5. Data were recorded continuously with a strip-chart recorder.

Each assay was performed at least twice, using different aliquots of the same preparation, except that isoelectric focusing and Cys modification used aliquots of different preparations. No significant differences were seen between replicate assays.

Results

Isoelectric Focusing. Both Pr and Pfr showed identical banding patterns after focusing (Figure 1). The p*I* range of 5.8–6.4 for isophytochromes is the same as that previously reported (Hunt & Pratt, 1979). After the 6-h focusing experiments, spectral assays of unstained gels indicated that no detectable thermal reversion of Pfr to Pr during focusing had occurred. Therefore, the absence of a difference between Pr and Pfr by this assay is not due to dark reversion of Pfr to Pr during focusing but instead indicates that Pr and Pfr have identical surface charges.

Histidine. Modification of His [34 residues per monomer (Hunt & Pratt, 1980)] showed that Pfr had 1.34/monomer modified immediately while Pr had 0.53/monomer modified immediately (Figure 2). The observed difference, 0.81, is actually 1.08 more His residues modified on Pfr since what one calls Pfr is really a photoequilibrium mixture of 75% Pfr and 25% Pr (Pratt, 1975). In addition, His residues in Pfr reacted slightly more rapidly than those in Pr. After 20-min reaction in either form, phytochrome was still fully photoreversible. Photoconversion of Pr to Pfr after a 2-min reaction resulted in an immediate modification of 0.95 more His/monomer (after accounting for the incomplete photoconversion described above; Figure 3a). Photoconversion of Pfr to Pr

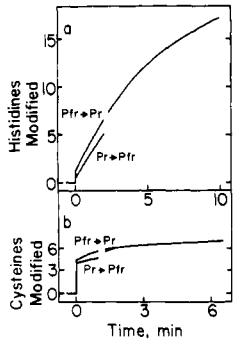

FIGURE 3: Effect of photoconversion on number of (a) His or (b) Cys residues per monomer modified. See legends to Figures 2 and 7 for conditions of assay. Gaps in the curves resulted from not recording during irradiations. After photoconversion of Pr to Pfr in (a), the two curves became coincident.

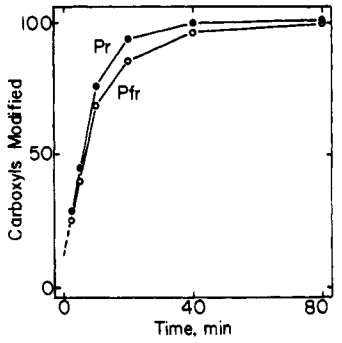

FIGURE 4: Number of carboxyl functions per monomer modified as a function of time in the presence of 33 mM 1-ethyl-3-[(dimethylamino)propyl]carbodiimide and 0.33 M [^{14}C]glycine ethyl ester, pH 4.5. Points are the means of duplicate determinations.

after 2 min, however, resulted in no immediate change in the number of His modified.

Carboxyl Functions. Modification of carboxyl functions [115 per monomer (Hunt & Pratt, 1980)] revealed that both Pr and Pfr have 12 functions that reacted immediately when the initial linear portion of the curve was extrapolated to zero time (Figure 4). The reaction of carboxyl functions on Pr proceeded slightly faster than it did for those on Pfr. Both curves are linear until about 74 functions are modified. Photoreversibility of these modified preparations could not be determined since undegraded phytochrome precipitated irreversibly at the acid pH used for modification.

Tyrosine. Both Pr and Pfr had 2 of the 23 total Tyr/monomer (Hunt & Pratt, 1980) modified immediately (Figure 5) after addition of a 110-fold molar excess of tetranitromethane. Tyrosines in Pr reacted more rapidly those of Pfr, but both reactions extrapolate to the same zero time value from any time point. This result further indicates that both forms of the molecule have the same number of surface Tyr.

Using the 110-fold molar excess of tetranitromethane over Tyr residues, both Pr and Pfr lose their photoreversibility within the first minute of reaction. However, lowering the amount of reagent to an 11-fold molar excess over Tyr and lowering the temperature to 0 °C slowed the reactions to measurable rates. Both forms of the molecule had one Tyr/monomer that reacted very rapidly (Figure 5, inset) and a second that reacted more slowly. After these two residues were modified, the reaction rates slowed dramatically, the Pfr rate even more than the Pr rate (data not shown) such that eventually more Tyr on Pr were modified as a function of time than were on Pfr (Figure 5).

Photoreversibility of phytochrome, measured under these latter conditions, declined rapidly to 50% of the initial level

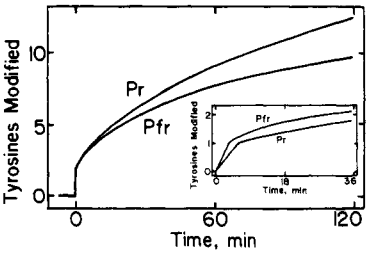

FIGURE 5: Number of Tyr residues per monomer modified as a function of time in the presence of a 110-fold molar excess of tetranitromethane over total Tyr at pH 8.0. Inset details the initial portion of reaction in the presence of an 11-fold molar excess of tetranitromethane over total Tyr. Data were recorded continuously with a strip-chart recorder.

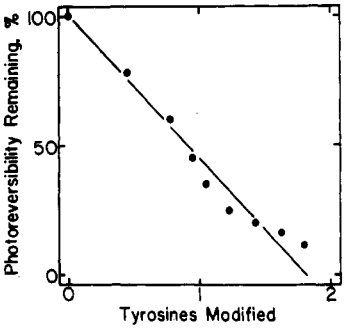

FIGURE 6: Loss of photoreversibility as a function of the number of Tyr residues per monomer modified. The number of Tyr residues modified is based on the average of the rates for Pr and Pfr since phytochrome is present in each form ~50% of the time during photoreversibility measurements. The line is a linear regression forced to 100% photoreversibility at zero modified Tyr residues.

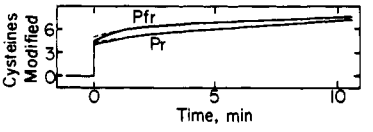

FIGURE 7: Number of Cys residues per monomer modified as a function of time in the presence of a 75-fold molar excess of dithionitrobenzoic acid over total Cys at pH 8.0. The dashed line is a tangent at 1 min to each curve. Data were recorded continuously with a strip-chart recorder.

after approximately 6 min and to below detection levels after 45 min. Plotting photoreversibility as a function of the number of Tyr modified (Figure 6) revealed that complete loss of photoreversibility occurred, within error, when an average of two Tyr/monomer was modified.

Reaction of Pr for about 8 min with a 50-fold molar excess of tetranitromethane at 0 °C yielded only a small effect on its extinction in the red spectral region. The absorption band of Pr at 667 nm was unchanged in peak wavelength and uniformly reduced in extinction by a maximum of 25%. Upon irradiation with red light, however, the sample became totally bleached between 500 and 800 nm. Reaction of Pr for 35 min with a 10-fold molar excess of tetranitromethane at 0 °C (compare to Figure 5, inset) produced the same results as just described, with total bleaching occurring within 45 s of the onset of a 30-s red actinic irradiation. Reaction of Pfr with a 50-fold molar excess of tetranitromethane at 0 °C resulted in its total bleaching between 500 and 800 nm within 6 min, with the Pr present remaining relatively unchanged as above. Reaction of Pfr with a 10-fold molar excess of tetranitromethane at 0 °C resulted in only a slow bleaching of Pfr, as monitored at 724 nm, with a time course similar to that for loss of photoreversibility shown in the inset of Figure 5.

Cysteine. Modification of Cys [14 per monomer (Hunt & Pratt, 1980)] revealed that Pfr had 4.55 freely available Cys

Table I

modified residue or function	numbers of residues modified immediately[a]			form modified faster	effect on photoreversibility
	Pr	Pfr	(Pfr − Pr)/0.75		
carboxyl	12[b]	12[b]	0	Pr	not determined[c]
histidine	0.53	1.34	1.08	Pfr	none
tyrosine	2.0[d]	2.0[d]	0	Pr	complete loss
	0[e]	0[e]	0	Pfr	lost during modification
cysteine	3.81[f]	4.55[f]	0.99	Pfr[g]	none

[a] Numbers are expressed on a per monomer basis. The difference is divided by 0.75 since Pfr is actually a mixture of 75% Pfr and 25% Pr. [b] Extrapolated to zero time for linear portion. [c] Not determined due to irreversible precipitation of phytochrome at pH (4.5) used for modification. [d] At 23 °C and 110-fold molar excess of reagent to Tyr content. [e] At 0 °C and 11-fold molar excess of reagent to Tyr content. [f] Extrapolated to zero time by tangent to curve at 1-min time point. [g] Reached 90% completion in 2.7 h for Pr and 5.7 h for Pfr.

(Figure 7) while Pr had 3.81, extrapolating both to zero time from 1 min. This difference quantifies an earlier report by Gardner et al. (1974) who used *N*-[^{14}C]ethylmaleimide and undegraded rye phytochrome. Correcting for the previously noted mixture of Pfr and Pr in the Pfr preparation, Pfr actually had 0.99 more freely available Cys, when extrapolating from 1 min to zero time. The largest actual difference that can be obtained from these curves is 1.8 more Cys in Pfr than Pr when extrapolating from 5 min to zero time. This difference declines, however, since remaining Cys on Pfr react more slowly than those on Pr. Pr reached 90% completion of the total reaction (14 Cys/monomer) in 2.7 h while Pfr required 5.7 h to reach the same level (data not shown). Neither Pr nor Pfr showed any loss of photoreversibility after reacting with dithiobis(nitrobenzoic acid) for 15 min. Photoconversion of Pr to Pfr after 1-min reaction resulted in rapid modification of 0.98 more Cys/monomer (accounting for imcomplete photoconversion; Figure 3b). Photoconversion of Pfr to Pr, however, indicated no rapid increase in the number of modified Cys.

Circular Dichroic Spectra. The far-ultraviolet CD spectra of the Pr and Pfr forms of undegraded oat phytochrome are essentially identical with those presented for undegraded rye phytochrome by Tobin & Briggs (1973). Both spectra show negative extrema at 220 nm and 209.5 nm. Using constrained least-squares analysis and standard reference CD spectra, we estimate phytochrome to contain 35% α helicity, 23% β structure, and 42% aperiodic structure. Small differences (±3%) in the ellipticities of Pr and Pfr between 206 to 221 nm were considered insignificant due to a ±10% noise level of $[\theta]_{mwr}$.

Discussion

The two forms of phytochrome, Pr and Pfr, though repeatedly photointerconvertible, are clearly different (Table I). Although Pr and Pfr are identical by isoelectric focusing and CD spectroscopy, Pfr has one more each His and Cys residues that reacted immediately with modifying reagents. Amino acid residues of Pfr reacted more slowly than those of Pr in two reactions (Cys and carboxyl modification), vice versa in one (His modification), and with changing rates in a third (Tyr modification). In addition, modification of an average of two Tyr residues per monomer resulted in complete loss of photoreversibility while modification of other residues (His, Cys) had no effect.

We recognize that all of the modifying reagents have side reactions with other amino acid residues under certain conditions. Since we employed the optimal conditions described for each reaction to minimize these complications, however, we assume that any side reactions were minimal. Because modification of the chromophore should produce a marked change in its visible extinction properties, the absence of any alteration in the magnitude of its photoreversible absorbance changes followed Cys and His modification indicates further that the chromophore was not altered.

Since Tyr modification did result in a total loss of photoreversibility (Figure 6), the possibility that the chromophore might be modified by tetranitromethane was examined experimentally. Again, since there was no change in the wavelength absorption maximum for Pr, we conclude that the modifying reagent did not chemically alter the chromophore. Tetranitromethane did result, however, in total bleaching of Pfr in darkness, indicating a possible selective modification of the chromophore in Pfr as opposed to Pr. That this bleaching was a consequence of the chemical modification of Tyr rather than the chromophore is established by the following observations. Total loss of photoreversibility (and extinction in the 500–800-nm region) was observed after incubation of Pr with a 10-fold molar excess of tetranitromethane for 35 min at 0 °C, followed by rapid transformation to Pfr. Under these same conditions, Pfr was modified only slowly, indicating that the loss of photoreversibility following incubation as Pr, which occurred within 45 s of the onset of the actinic red light, did not occur because of a rapid modification of Pfr following photoconversion. Because Pfr bleached in the absence of any actinic irradiation, these data indicate that the first two Tyr residues that are modified are needed to maintain the Pfr form of phytochrome.

Two postulates exist to explain differences between Pr and Pfr. The first is that photoconversion causes a protein conformational change that leads to photomorphogenic activity [cf. Smith (1975) and Pratt (1979) for discussions]. A more recent postulate by Song et al. (1979) is that photoconversion causes the chromophore to become more flexible in Pfr with respect to its binding to the protein moiety, making available a new portion of the surface of the protein but not resulting in a conformational change. The data presented here more strongly support the former postulate. Both Pr and Pfr have identical secondary structure, as measured by circular dichroism. Pfr had more freely available amino acids in two experiments than did Pr, but Pr did not have more than Pfr in any of these experiments. While these data are consistent with both postulates, only a protein conformational change could account easily for the observed differences in reaction rates between the two forms. Movement of the chromophore, by itself, while resulting in increased availability of *some* residues to the solvent, cannot explain the different reaction rates that involve *most* of the residues. Futhermore, addition of bulky side groups to amino acids that are reversibly exposed might be expected to inhibit photoreversibility in the chromophore movement model. While the number of His and Cys residues, however, is increased by photoconversion of Pr to Pfr, photoreversibility of either form is not affected by modification

of those reversibly available residues. The protein conformational change model can accommodate this potential problem if the modified residues that are reversibly exposed to the solvent are not critical to either conformation but simply reflect the change.

Photoconversion of Pr to Pfr *during* the His and Cys modification reactions resulted in a rapid increase, by one each, in the number of residues modified (Figure 3). However, photoconversion of Pfr to Pr had no detectable effect. These results indicate that the difference of one residue each per monomer (Figure 2, 7) is due to exposure of one His or one Cys/monomer to the solvent upon photoconversion of Pr to Pfr. The results rule out more complicated explanations such as exposure of two residues/monomer while one is covered upon photoconversion.

While it is tempting to conclude that these data prove the conformational change hypothesis, another explanation is also possible. Phytochrome, as Pr, may be an open, floppy molecule existing in a variety of conformations. Photoconversion to Pfr may stabilize phytochrome in a particular conformation, perhaps one not previously accessible to Pr. Such a mechanism has been postulated for a variety of polypeptide hormones from mammalian sources (Schwyzer, 1968). This postulate suggests that amino acid residues in Pr should be more accessible to modifying reagents, as is the case for all reactions except His modification and the first two Tyr/monomer modifications, than those in Pfr. The His exception may be accounted for if photoconversion of Pfr to Pr results in burying of some His residues inside the molecule, making them less accessible to the modifying reagent.

The data presented here describe some of the chemical differences between Pr and Pfr, extending previous analyses (Tobin & Briggs, 1973; Gardner et al., 1974; Lisansky & Galston, 1974, 1976; Pratt & Cundiff, 1975). However, we must emphasize that, while these data support postulates concerning phytochrome structural changes, they are not conclusive. Resolution of any structural differences between Pr and Pfr will require detailed structural analysis.

Acknowledgments

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References

Cundiff, S. C., & Pratt, L. H. (1975) *Plant Physiol. 55*, 207–211.

Gardner, G., Pike, C. S., Rice, H. V., & Briggs, W. R. (1971) *Plant Physiol. 48*, 686–693.

Gardner, G., Thompson, W. F., & Briggs, W. R. (1974) *Planta 117*, 367–372.

Habeeb, A. F. S. A. (1972) *Methods Enzymol. 25*, 457–464.

Hoare, D. G., & Koshland, D. E., Jr. (1967) *J. Biol. Chem. 242*, 2447–2453.

Hunt, R. E., & Pratt, L. H. (1979) *Plant Physiol. 64*, 332–336.

Hunt, R. E., & Pratt, L. H. (1980) *Biochemistry 19*, 390–394.

Kidd, G. H., & Pratt, L. H. (1973) *Plant Physiol. 52*, 309–311.

Lisansky, S. G., & Galston, A. W. (1974) *Plant Physiol. 53*, 352–359.

Lisansky, S. G., & Galston, A. W. (1976) *Plant Physiol. 57*, 188–191.

Miles, E. W. (1977) *Methods Enzymol. 47*, 431–442.

Otavsky, W. I., & Drysdale, J. W. (1975) *Anal. Biochem. 65*, 533–536.

Pratt, L. H. (1973) *Plant Physiol. 51*, 203–209.

Pratt, L. H. (1975) *Photochem. Photobiol. 22*, 33–36.

Pratt, L. H. (1979) *Photochem. Photobiol. Rev. 4*, 59–124.

Pratt, L. H., & Cundiff, S. C. (1975) *Photochem. Photobiol. 21*, 91–97.

Pratt, L. H., & Marmé, D. (1976) *Plant Physiol. 58*, 686–692.

Rice, H. W., & Briggs, W. R. (1973) *Plant Physiol. 51*, 939–945.

Righetti, P., & Drysdale, J. W. (1971) *Biochim. Biophys. Acta 236*, 17–28.

Schwyzer, R. (1968) *J. Mond. Pharm. 3*, 254–263.

Smith, H. (1975) *Phytochrome and Photomorphogenesis*, McGraw-Hill, London.

Sokolovsky, M., Riordin, J. F., & Vallee, B. L. (1966) *Biochemistry 5*, 3582–3589.

Song, P. S., Chae, Q., & Gardner, J. D. (1979) *Biochim. Biophys. Acta 576*, 479–495.

Tobin, E. M., & Briggs, W. R. (1973) *Photochem. Photobiol. 18*, 487–495.