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Molecular Topography of Phytochrome As Deduced from the Tritium-Exchange Method†

Tae-Ryong Hahn and Pill-Soon Song*

ABSTRACT: The hydrogen–tritium-exchange measurements on phytochrome have been performed to detect the conformational differences between the red-absorbing (Pr) and the far-red-absorbing (Pfr) forms of phytochrome. The large and small Pfr molecules revealed more exchangeable protons than did the corresponding Pr molecules by 96 and 70 protons, respectively. These results suggest that the Pr → Pfr phototransformation is accompanied by an additional exposure of the peptide chains in the Pfr molecule. Of 1682 theoretically exchangeable hydrogens in undegraded phytochrome, only 442 (26%) and 346 (21%) protons were found to be exchangeable (excluding instantaneously exchangeable protons that cannot be determined by the present method). Thus, the phytochrome protein appears to be compact and highly folded. The kinetic analyses of the tritium exchange-out curves indicate that two kinetically different groups are responsible for the conformational differences between the Pr and Pfr forms of phytochrome. These components are due to (1) the exposure of hydrogen-bonded peptide segments (α helix and/or β-pleated sheet) in the chromophore vicinity of Pfr and (2) the exposure of hydrogen-bonded peptide segments on the chromophore peptide domain as well as on the chromophore-free tryptic domain of undegraded phytochrome.

Phytochrome mediates a variety of the morphogenic and developmental responses of higher plants to red light. There are two forms of phytochrome, an inactive red-absorbing form (Pr)[1] and an active far-red-absorbing form (Pfr). The latter is formed from the former by red light [see reviews by Kendrick & Spruit (1977), Pratt (1978), and Rüdiger (1980)].

From detailed spectroscopic analyses of the absorption spectra of Pr and Pfr, it was concluded that the chromophores of both phytochrome forms possess largely similar conformations, excluding a *gross* isomerization of the chromophore

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[1] Abbreviations: EDTA, ethylenediaminetetraacetic acid; Pfr, far-red-absorbing form of phytochrome; Pr, red-absorbing form of phytochrome; $NaDodSO_4$, sodium dodecyl sulfate; ANS, 8-anilinonaphthalene-1-sulfonate.

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in the Pr → Pfr phototransformation (Song et al., 1979; Song & Chae, 1979). Further, no significant differences between the two forms of phytochrome were found in size, shape, or ionic charges of the apoprotein [Tobin & Briggs, 1973; Song et al., 1979; Hunt & Pratt, 1981; see reviews by Pratt (1978) and Song (1980, 1982)]. However, other properties were noticeably different, including lower solubility (Pratt, 1978; Song, 1980), lower induced CD of the $Q_{x,y}$ bands (Song et al., 1979), and higher susceptibility to ammonium sulfate, urea, metal ions, glutaraldehyde, and sulfhydryl reagents for Pfr than for Pr [see reviews by Pratt (1978) and Song (1980)]. Some of the amino acid residues are also more accessible to chemical modifying agents in the Pfr form than in the Pr form (Gardner et al., 1974; Hunt & Pratt, 1981).

To provide a working hypothesis for the molecular basis of the physiologically active, Pfr, form of phytochrome, we proposed a model that effectively accommodates most of the above-mentioned observations (Song et al., 1979; Song, 1980). The model features (i) a rigidly held chromophore in the Pr crevice, (ii) the reorientation of the chromophore, (iii) and the exposure of a hydrophobic protein crevice upon phototransformation of Pr to Pfr. Several additional lines of evidence in support of the proposed model have been obtained in terms of the reorientation of the chromophore (Sarkar & Song, 1981), the preferential exposure of the chromophore (Hahn et al., 1980), and the generation of a hydrophobic surface on the Pfr protein (Hahn & Song, 1981; Kim & Song, 1981; Smith & Daniels, 1981a; Yamamoto & Smith, 1981). A recent 360-MHz NMR study of Pr and Pfr also provided additional support for the hydrophobic model of Pfr (H. K. Sarkar, P.-S. Song, H. Tabba, and K. M. Smith, unpublished experiments; Song, 1982).

Although the differences between the Pr and Pfr forms of phytochrome are qualitatively apparent, as has been recently summarized (Song, 1982), it is desirable to establish the differences between them on a quantitative basis. For this purpose, we adopt the tritium-exchange method.

Hydrogens bound to oxygen, nitrogen, and sulfur atoms in protein can be exchanged with tritiums in a medium containing radioactive tritium oxide [Englander, 1968; see Englander & Englander (1978) and references cited therein; Liem et al., 1980]. In contrast to other chemical reactivity measurements, such as the chemical modifications of functional differences of functional groups (Hunt & Pratt, 1981), this method is particularly useful in detecting the conformational differences of phytochromes, since no significant alteration of the conformation is induced by the tritium exchange.

In this paper, we report the conformational differences between the Pr and Pfr forms of phytochrome in terms of tritium exchanges. From the kinetic analysis of the exchange data, it has also been possible to deduce the molecular topography of phytochrome. Finally, the tritium exchange data have provided complimentary evidence in support of the hydrophobic model of the physiologically active form (Pfr) of phytochrome.

Materials and Methods

Undegraded, large phytochrome (molecular weight ~ 120000) was isolated and purified by the Affi-gel Blue affinity chromatographic technique from etiolated oat seedlings (*Avena sativa* L., CV Garry), as described previously (Smith & Daniels, 1981b; Song et al., 1981). Briefly, 2.4 kg of dark-grown oat tissues was extracted with 50 mM Tris buffer, pH 8.5, containing 1.4% mercaptoethanol and was chromatographed through a brushite column (11 × 13 cm) at a flow rate of 1.5 L/h. A 150–200 mL sample of the phytochrome fraction was fractionated with 30% ammonium sulfate. About 20 mg of the phytochrome fractions was then applied on an Affi-gel Blue column (2.5 × 30 cm), which was equilibrated in 0.1 M potassium phosphate buffer, pH 7.8, containing 0.1% mercaptoethanol. After the column was washed with 0.1 M phosphate buffer, pH 7.8, containing 0.5 M KCl and 0.1% mercaptoethanol, the phytochrome-bound Blue dye was eluted with the same starting buffer containing 10 mM lumichrome-free flavin mononucleotide purified successively with Bio-Bead SM-2 (Bio-Rad) and a Sephadex G-15 column (2 × 40 cm) as described elsewhere (Song et al., 1981), followed by fractionation with an equal volume of saturated ammonium sulfate solution (pH 7.8). Phytochrome pellets were redissolved in 0.1 M sodium phosphate buffer, pH 7.8, 50 mM KCl, and 0.1 mM EDTA, and the solution was subjected to a Bio-Gel 0.5-m column (2.5 × 90 cm) and chromatographed with the same buffer at a flow rate of 30 mL/h. In the final preparation, 8–10 mg of purified phytochrome with a specific absorbance ratio of 0.8 (A_{660}/A_{280}) was obtained.

The small molecular weight phytochrome (60000) was also purified by Affi-gel Blue affinity chromatography of the tryptic digest of the large molecular weight phytochrome. The molecular weight of the former was determined by NaDodSO$_4$–polyacrylamide gel electrophoresis.

All chemicals including Sephadex G-25-150 were purchased from Sigma Chemical Co., St. Louis. Tritiated water was obtained from New England Nuclear Co. The specific activity was 100 mCi/g and was used at dilutions of approximately 10-fold. All buffers in the purification and solution preparations of phytochrome were made with deionized, redistilled water. Unless otherwise specified, 0.1 M sodium phosphate buffer, pH 7.8, containing 50 mM KCl and 0.1 mM EDTA was used for solutions and column operations.

The method of gel-permeation chromatography was adopted to follow both exchange-in and exchange-out rates of hydrogen–tritium exchanges in phytochrome (Englander, 1963, 1968). The exchange-in measures the number of tritium ions incorporated into the protein, while the exchange-out measures the rate of exchange-out of the bound tritium. Through a preliminary experiment with ribonuclease, appropriate column size and packing materials were determined in terms of resolution and efficiency of the gel-permeation chromatography.

In exchange-in measurements, 2.0 mL of large phytochrome (A_{660} = 0.2; 2.9 μM) in 0.1 M sodium phosphate buffer, pH 7.8, containing 50 mM KCl and 0.1 mM EDTA, was mixed with 0.2 mL of tritiated water (specific activity 100 mCi/g) and incubated. During incubation, 0.25 mL of the aliquots was taken at various time intervals and applied to a G-25 column (1.0 × 12.0 cm). The column was eluted with the same buffer. The absorbances at 660 nm for Pr and 723 nm for Pfr were immediately recorded for the collected effluent fractions (0.5 mL/tube). A 0.1-mL sample of each fraction was counted for radioactivity. Figure 1 shows a typical profile for the Pr solution incubated in THO for 2 h, following the above procedure. The ratios of radioactivity (in cpm) to absorbance through the peak region are shown in the inset of Figure 1, as the amount of exchanged-in tritium should be proportional to the protein concentration by a constant value (Englander, 1963). Each of four fractions yielded essentially the same value, indicating that the separation was satisfactory. The average of the upper three values was used for the calculation of the number of exchanged hydrogens per molecule of protein.

The kinetics of exchange-out were followed by both one-column and two-columns separation procedures. For one-

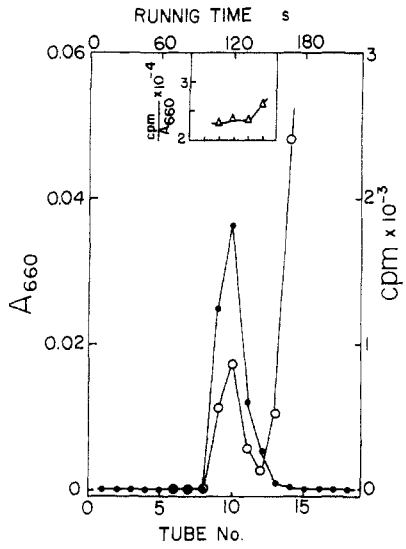

FIGURE 1: Gel filtration profile of phytochrome (Pr) incubated in tritiated water (10 mCi/mL) for 2 h. A Sephadex G-25-150 column (1.0 × 12.0 cm) was used for the separation of tritiated phytochrome from THO. Incubated Pr (0.25 mL) was applied to the column and eluted with 0.1 M phosphate buffer, pH 7.8, containing 0.1 mM EDTA and 50 mM KCl at a flow rate of 2.5 mL/min. The collected fractions (0.5 mL/tube) were measured for the absorbance at 660 nm (●), and a 0.1-mL aliquot of each fraction was counted for radioactivity (○). The inset shows the ratio of counts per minute to absorbance over the peak region.

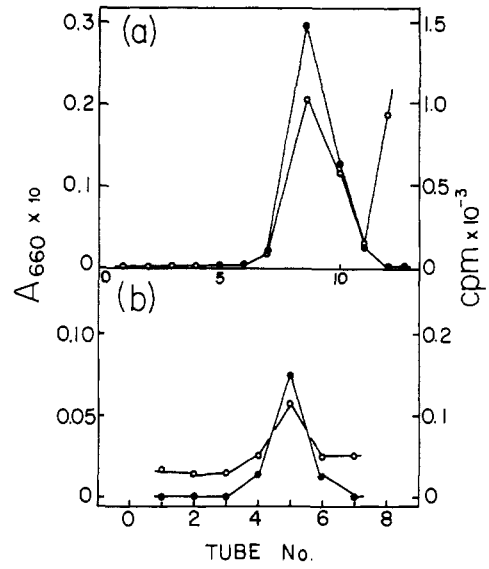

FIGURE 2: Two-column method for exchange-out for Pr. (a) Gel filtration profile of the first G-25-150 column (2.5 × 9.0 cm). Pr (1.4 mL) incubated in tritiated water (10 mCi/mL) for 3 days was applied to the column and eluted with phosphate buffer, pH 7.8, at a flow rate of 8.0 mL/min. The collected fractions (2.0 mL/tube) were measured for the absorbance at 660 nm (●) and radioactivity (○). Two major fractions (no. 8 and no. 9 tubes) were pooled and used for exchange-out experiments. (b) Gel filtration profile of the second G-25-150 column (1.0 × 12.0 cm) for Pr, 6 h of exchange-out. A 0.5-mL aliquot was taken from the pooled fraction and passed through a second column at a flow rate of 2.5 mL/min. A 1.0-mL sample of the effluent was collected per tube for absorbance measurements (●) and radioactivity (○).

column separation, the solution prepared by the same procedure used for the exchange-in experiment and incubated for 3 days at 278 K (to establish equilibrium) was subjected to a G-25 column (1 × 12 cm). When the solution of phytochrome passed about halfway through the column, the flow was stopped to allow exchange-out of bound tritium in the medium. At any given time interval, the column operation was resumed. The collected fractions were then measured for radioactivity and absorbance. The limitation of the incubation time in one-column methods is determined by the loss of chromatographic resolution due to the diffusion of background tritium into the protein fraction (Englander, 1963). The one-column method was used only for the exchange-out rate measurement of the incubation time period within 30 min to avoid this limitation. For the exchange measurements of longer incubation times, the two-column method was employed. A 1–2 mL sample of the phytochrome solution, prepared and incubated in the same manner as for the one-column exchange-out separation, was chromatographed first through a G-25 column (2.5 × 9.0 cm). After the absorbances of the collected fractions were checked (each fraction, 2 mL/tube), two major fractions containing phytochrome were pooled and incubated for given time intervals. A 0.5-mL sample was taken from the aliquots and passed through a second column (1 × 12 cm) to remove unbound tritiums, which were exchanged-out during incubation. The radioactivity and absorbances of the collected fractions (1 mL/tube) were then recorded. A typical elution profile is shown in Figure 2. Both first and second columns gave a good resolution.

The exchange-in and -out data for the small molecular weight phytochrome were obtained in the same way as for the large molecular weight phytochrome, except lower concentrations of phytochrome (A_{660} = 0.1) and tritiated water (0.1 mL of tritiated water with a specific activity of 100 mCi/g) were used per 2 mL of phytochrome.

All incubation and tritium-exchange operations were carried out in a cold chamber (278 K) under safe green light. The Pfr form of phytochrome was prepared by red light irradiation of a Pr solution with a Bausch & Lomb microscope illuminator (660-nm interference filter; fluence rate at 7.5 W/m^2). In order to prevent the dark reversion of Pfr, the Pfr solution was continuously irradiated during the period of the exchange experiment, except for the short duration of column operation (cf. Figure 1). For one-column, exchange-out measurements, the middle section of the column where Pfr is located was irradiated with the same source of red light. The absorbance of each fraction was recorded on a Cary 118C spectrophotometer. The concentrations of Pr and Pfr were calculated from the reported extinction coefficients of phytochrome (Tobin & Briggs, 1973).

The radioactivity of each fraction from a gel-permeation column was counted in a xylene-based scintillation fluid containing 0.3% 2,5-diphenyloxazole, 0.02% 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene, and 25% Triton X-114 with a Beckman L200 liquid scintillation counter. At least three independent chromatographic runs were made to obtain a mean data point for both exchange-in and -out measurements. The total number of exchangeable hydrogens were calculated as follows:

$$^3\text{H per molecule} = 111(C/C_0)(\epsilon_P/A) \qquad (1)$$

where C_0 is the initial tritium activity in the protein–tritiated water mixture, C is the tritium activity carried by the protein, as counted in the column effluent, and ϵ_P and A are the molar extinction coefficient and absorbance of the protein in the column effluent, respectively. The numerical constant of 111 is the atom concentration of hydrogen in water.

Results

Figure 3 shows that hydrogen–tritium exchange is significantly greater with Pfr than with Pr for both large and small molecular weight phytochromes. The number of hydrogen–tritium exchanges for the small molecular weight phytochrome

Table I: Half-Life ($t_{1/2}$) and Number of Exchanged Hydrogens per Molecule for Each Kinetic Component, Evaluated from Exchange-Out Curves of Phytochrome

exchange group		small phytochrome			large phytochrome		
		Pr	Pfr	Δ^3H_{ex}[a]	Pr	Pfr	Δ^3H_{ex}[b]
I	$t_{1/2}$ (min)	6.2 ($\bar{r}$ = 1.00)[b]	7.9 ($\bar{r}$ = 0.959)		6.0 ($\bar{r}$ = 1.00)	7.3 ($\bar{r}$ = 0.997)	
	^{3}H/molecule	59 ± 3	90 ± 3	31	118 ± 3	181 ± 9	63
II	$t_{1/2}$ (h)	2.0 ($\bar{r}$ = 0.975)	2.5 ($\bar{r}$ = 0.993)		1.6 ($\bar{r}$ = 0.997)	1.5 ($\bar{r}$ = 0.992)	
	^{3}H/molecule	75 ± 5	108 ± 6	33	148 ± 9	180 ± 10	32
III	$t_{1/2}$ (h)	88 ($\bar{r}$ = 1.00)	79 ($\bar{r}$ = 1.00)		62 ($\bar{r}$ = 1.00)	54 ($\bar{r}$ = 1.00)	
	^{3}H/molecule	34 ± 2	40 ± 3	6	80 ± 6	81 ± 7	1
	total ^{3}H/molecule	168	238	70	346	442	96

[a] $\Delta^3H_{ex} = {}^3H_{Pfr} - {}^3H_{Pr}$, where ${}^3H_{Pr}$ and ${}^3H_{Pfr}$ represent the number of exchanged hydrogens in Pr and Pfr, respectively. [b] $\bar{r}$ is the correlation coefficient.

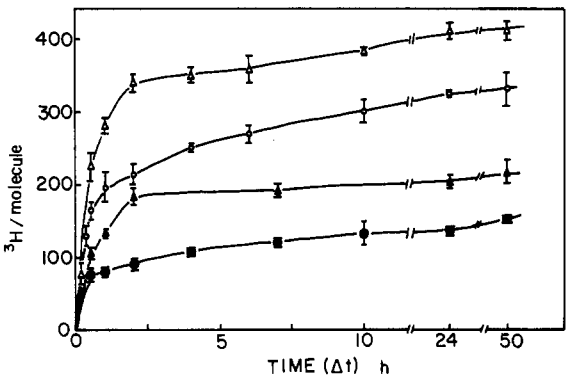

FIGURE 3: Tritium exchange-in curves for small [Pr (●), Pfr (▲)] and large [Pr (○), Pfr (△)] molecular weight phytochromes at 278 K in 0.1 M sodium phosphate buffer, pH 7.8, containing 0.1 mM EDTA and 50 mM KCl. The number of ^{3}H exchanged, ^{3}H/molecule, was calculated by using eq 1.

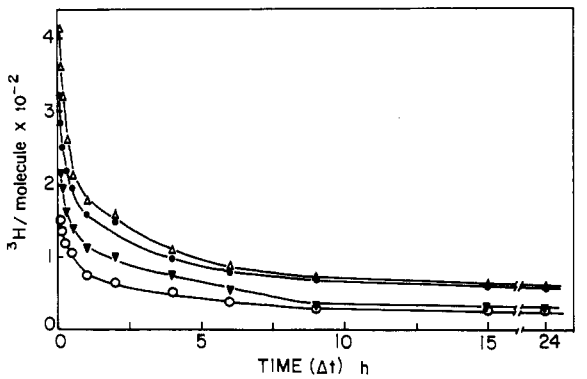

FIGURE 4: Tritium exchange-out curves (back-exchange) of small [Pr (○), Pfr (▼)] and large [Pr (●), Pfr (△)] molecular weight phytochromes at 278 K against 0.1 M sodium phosphate buffer, pH 7.8, containing 0.1 mM EDTA and 50 mM KCl. The number of ^{3}H exchanged, ^{3}H/molecule, was calculated by using eq 1.

is approximately half that of the large molecular weight phytochrome in both Pr and Pfr forms, during all stages of exchange-in. After a long incubation period (50 h) when the exchange is at equilibrium, the differences (per molecule) in the total number of exchanged hydrogens between the Pr and Pfr forms are approximately 100 and 70 for large and small phytochromes, respectively. This result suggests that the majority of exchangeable hydrogens arise from the chromophore-bearing part (60000 daltons) of the large phytochrome molecule (120000 daltons).

After a 30-min incubation, about half of the total exchangeable hydrogens were exchanged-in for all four cases (Figure 3). After the initial rapid exchange, the exchange-in rate slowed, as the exchange approached equilibrium. After a 24-h incubation, only a very small increase in the exchange-in was observed. Both Pr and Pfr showed essentially the same exchange kinetic behaviors, except for the first 30-min to 2-h incubation period, where the exchange-in rate of Pr started to level off at lower values than did the Pfr rate (Figure 3). The small molecular weight phytochrome showed almost identical kinetic behaviors as the large molecular weight phytochrome.

Quantitative analysis of these exchange-in kinetics is difficult, since the concentration of tritium used was much less than that of water, and thus, the tritium ions exchanged-in are immediately exchanged-out. On the other hand, the kinetic analysis of the exchange-out curves (Figure 4) can be performed, since the concentration of water exceeds that of the bound tritium.

The exchange-out kinetic curves for large and small phytochromes in 0.1 M sodium phosphate buffer, pH 7.8, containing 50 mM KCl and 0.1 mM EDTA at 278 K are shown in Figure 4. Analogous to the exchange-in data shown in Figure 3, all four exchange-out curves follow an initial rapid drop followed by a gradual leveling off.

Exchange-out kinetics are first order if the environments of the exchangeable protons in a native protein are homogeneous. This is rarely the case. There are generally several kinetic groups of exchangeable hydrogens in a native protein [Englander, 1963; see Englander & Englander (1978) and references cited therein]. A group of hydrogens is usually lost before the earliest measurement can be made ("instantaneous group"). Group I hydrogens exchange relatively rapidly (half-life in minutes) compared to group II hydrogens which have an apparent half-life of several hours. Group III hydrogens exchange very slowly.

The complicated exchange-out kinetics of phytochrome (Figure 4) can be approximately resolved, in terms of several first-order components with different exchange-out half-lives, with the aid of the peeling procedure (Van Liew, 1967; Hahn & Song, 1981). From the first-order plots of the data shown in Figure 4, the slowest component straight line (correlation coefficients greater than 0.98) was extrapolated to the ordinate where the intercept and slope represent the magnitude and rate constant of the component, respectively. If the exchange-out processes were of kinetics of two first order, the subtraction of the extrapolated line (slowest component) from the original curve would have resulted in a straight line. This was not the case (Figure 5), suggesting that there are more than two first-order components in the original plot. Thus, the same peeling procedure was applied to the data shown in Figure 5, resulting in the appearance of the additional kinetic component (inset). We labeled the fast (Figure 5, inset), intermediate (Figure 5), and slow (plots not shown) components groups I, II, and III, respectively. The results of these analyses are presented in Table I.

Group I exchangeable hydrogens show half-lives of 6–8 min, whereas groups II and III exchange-out hydrogens more

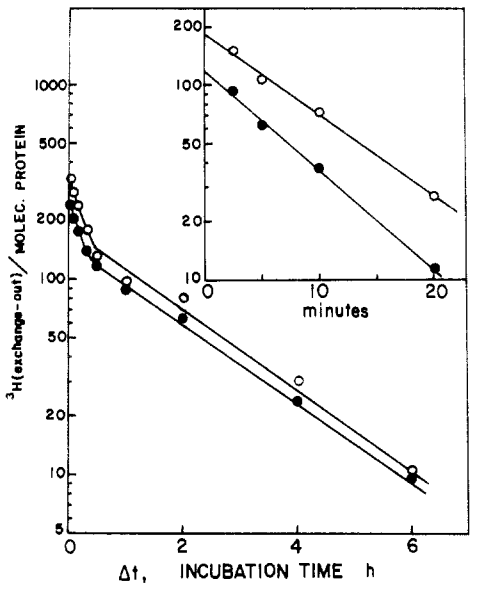

FIGURE 5: Peeling plots for group I and II exchangeable tritiums (exchange-out) after subtracting the group III kinetic component from the first-order plots of exchange-out curves from Figure 4, for large molecular weight Pr (●) and Pfr (○). The straight lines were derived from the linear regression analysis. The inset shows the peeling plots after subtracting the group II kinetic components from the plots shown in the main panel. See text and Hahn & Song (1981) for details of the peeling procedure. The number of ^{3}H exchanged, ^{3}H(exchanged-out)/molecule, was calculated by using eq 1.

slowly, with half-lives of 1.5–2.5 h and longer than 50 h, respectively. Differences in the total number of the exchangeable hydrogens between Pr and Pfr are 96 and 70 for the large and small molecular weight phytochromes, respectively. This result is consistent with the exchange-in data. Of the additional 96 exchangeable hydrogens arising from the Pr → Pfr phototransformation of the large phytochrome, 63 and 32 hydrogens are attributable to groups I and II, respectively. On the other hand, of the additional 70 exchangeable hydrogens from the phototransformation of the small phytochrome, 31 and 33 hydrogens belong to groups I and II, respectively. These results indicate that, upon Pr → Pfr transformation, the major structural changes in the phytochrome molecule are reflected in group I and II peptide segments of the large molecular weight phytochrome. However, it is clear from Table I (cf. ΔH_{ex} for small and large phytochromes) that the group I hydrogen exchanges arise from both chromophore as well as nonchromophore moieties, while the group II hydrogen exchanges occur only in the chromophore-bound peptide segment (equivalent to the small molecular weight phytochrome).

Discussion

The total number of exchangeable hydrogens obtained from both exchange-in and exchange-out measurements is greater for Pfr than for Pr in both large and small molecular weight phytochromes (Figures 3 and 4, Table I), indicating that the Pr → Pfr phototransformation exposes additional peptide segments of the protein moiety.

From the known amino acid composition data for small (Mumford & Jenner, 1966) and large oat phytochromes (Hunt & Pratt, 1980), it is possible to calculate the total number of exchangeable hydrogens in a completely unfolded conformation. These values are 892 and 1682, respectively. Of 892 hydrogens, 238 (27%) are actually exchangeable in the small Pfr phytochrome, while 442 (26%) out of 1682 hydrogens are exchangeable in the large Pfr phytochrome. For the large and small Pr molecules, the exchangeable hydrogens (346 and 168, respectively) represent 21 and 19% of their theoretical values, respectively (Figure 3 and Table I). These results suggest that the apoproteins of both small and large phytochromes are compact and highly folded.

The total number of exchangeable protons in small Pr is approximately half that of large Pr, indicating that the exposed surface area of the former is about half that of the latter. This result can be accommodated in terms of the apoprotein consisting of two globular domains (ca. 60 000 daltons each) or the "dumbbell"-shaped phytochrome molecule (Smith, 1975; Rüdiger, 1980) derived from electron microscopic data (Correll et al., 1968; Briggs, 1972).[2] The small molecular weight phytochrome then represents one domain or lobe of the dumbbell-shaped molecule, as the result of tryptic cleavage. According to the molecular shape, a large change in the total surface area (two lobes or domains) is not expected in going from the large to the small molecular weight phytochrome, if the tryptic cleavage occurs at the connecting peptide segment between the dumbbell lobes or peptide domains.

Characteristic half-lives of peptide protons in hydrogen-bonded peptides can vary from a few minutes to hours at room temperature [Englander, 1963; see Englander & Englander (1978) and references cited therein]. It is thus reasonable to assign group I exchangeable hydrogens (Table I) to the exposed hydrogen-bonded peptide hydrogens with half-lives of 6–8 min at 5 °C. Group II and III hydrogens can be assigned to the hydrogen-bonded protons sterically blocked from direct contact with water [see Englander & Englander (1978) and references cited therein]. Group II exchangeable hydrogens with a half-life of 1.5–2.5 h represent the α-helical and/or β-pleated sheet peptide protons in somewhat exposed segments,[3] whereas group III hydrogens can be assigned to shielded α-helical and β-chain peptide hydrogens. These hydrogens exchange only extremely slowly with a half-life of 3–4 days, particularly under the condition of relatively low temperature at 278 K. Thus, a relatively small number of group III hydrogens are exchanged, and there is no significant difference in group III hydrogens seen between the Pr and Pfr forms of phytochrome (Table I).

From Table I, it can be seen that the value of the difference (ΔH_{ex}) in group I hydrogens between the small Pr and Pfr molecules is almost exactly half of those for the large phytochromes. Thus, it can be suggested that the tryptic hydrolysis cleaves the fully exposed hydrogen-bonded peptide segment into approximately equal parts. On the other hand, the ΔH_{ex} values in group II hydrogens are identical for both the large and small phytochromes; i.e., the tryptic moiety (60 000 daltons) without the chromophore does not contribute to the group II exchange-out, as the exchange appears to be confined within the chromophore-carrying protein moiety (i.e., small molecular weight phytochrome). It should be noted here that there is no significant unfolding of the secondary structure of the large molecular weight protein upon Pr → Pfr transformation, as judged by the CD spectra (Tobin & Briggs, 1973; Song et al., 1979).

From the above discussion, the molecular topography of Pr and Pfr molecules can be described as follows. There are two domains of the polypeptide chains which become preferentially exposed on the Pfr molecule, as revealed by the group I and II exchange-out rates. Since the group II exchangeable hy-

[2] The electron micrographs of rye photochrome do not clearly reveal the shape of the 120 000-dalton phytochrome (Smith & Correll, 1975).

[3] Three hydrogens on the pyrrolic rings of the chromophore, which becomes exposed upon Pr → Pfr conversion, may also contribute to this group (Song et al., 1979; Hahn et al., 1980).

drogens remain the same for the small and large molecular weight phytochromes, the group II exchange-out can be assigned either to the chromophore and crevice area which becomes more exposed on the Pfr protein (Hahn et al., 1980; Hahn & Song, 1981) or to an area removed from the chromophore crevice on one (i.e., small Pfr) of the two dumbbell-shaped lobes of the large phytochrome molecule. Pratt (1982) proposes a conformational change away from the chromophore crevice. As for the former assignment, there are several lines of evidence consistent with the concept of exposure of the chromophore binding proper in terms of the effects of ANS (Hahn & Song, 1981) and deuterium oxide on the photoreversion and dark reversion of Pfr and chromophore hydrogen–deuterium exchange on Pfr (Sarkar & Song, 1981) and in terms of the alteration of the relative orientation of the chromophore with respect to the proximal tryptophan residue upon Pr → Pfr transformation (Song, 1982; Sarkar & Song, 1982). A distal conformation change, however, is also possible through cooperative interactions between the helical domains of the apoprotein induced by the changes at and near the chromophore crevice. However, a precedence for an exclusive distal conformation change without altering the ligand (or chromophore) binding site has yet to be elucidated by the crystallographic method.

In conclusion, one of the critical changes accompanying the Pr → Pfr phototransformation is that parts of the α-helical and/or β-sheet peptide segments become exposed. A recent 360-MHz NMR study also reveals the exposure of several amino acid residues on the Pfr protein (H. K. Sarkar, P.-S. Song, H. Tabba, and K. M. Smith, unpublished experiments). One or more of the exposed basic amino acid residues also appears to be responsible for the pH increase of unbuffered phytochrome solution upon Pr → Pfr phototransformation (Tokutomi et al., 1982).

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