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A Photoreversible Circular Dichroism Spectral Change in Oat Phytochrome Is Suppressed by a Monoclonal Antibody That Binds near Its N-Terminus and by Chromophore Modification†

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ABSTRACT: Accompanying the phototransformation of native 124-kilodalton (kDa) oat phytochrome from red-absorbing form (Pr) to far-red-absorbing form (Pfr), there is a photoreversible change in circular dichroism (CD) in the far-UV region indicative of a 3% increase in α-helical folding of apoprotein. To elucidate the conformational change involved in the phytochrome phototransformation, several monoclonal antibodies have been used as epitope-specific probes. Monoclonal antibody oat-25 suppressed the photoreversible CD spectral change using phytochrome with an A_{666}/A_{280} as Pr of 1.13. Monoclonal antibodies oat-22, oat-13, and oat-31 did not significantly affect the CD spectral change of phytochrome. Oat-25 requires an epitope near the N-terminus of phytochrome. Oat-22, oat-13, and oat-31 recognize epitopes on the N-terminus, chromophore-containing half of phytochrome, albeit further removed from the N-terminus than that recognized by oat-25. Interestingly, oat-13 and oat-31 did, however, induce a time-dependent decrease in the far-UV CD, apparently due to aggregation of phytochrome (both Pr and Pfr forms). Monoclonal antibodies oat-26 and oat-28, which recognize epitopes on the C-terminus half of phytochrome, also did not suppress the photoreversible CD change, although oat-26 and oat-28 slightly inhibited it. The photoreversible CD spectral change can also be inhibited by sodium borohydride, which bleaches the chromophore by reducing it, and by tetranitromethane, which oxidizes the chromophore of phytochrome. Although explanations of these results based on indirect interactions between the chromophore and the N-terminus segment are possible, we propose that an additional α-helical folding of the Pfr form of the phytochrome may result from a photoreversible interaction between the Pfr form of the chromophore and the N-terminus segment.

Phytochrome is a blue-green chromoprotein that serves as the photoreceptor for a variety of morphogenic and developmental responses in plants, according to the following photoreversible scheme (Scheme I) [for recent reviews, see Pratt (1982), Smith (1983), and Lagarias (1985)].

Scheme I

$$\text{Pr (inactive form)} \underset{\text{730-nm light}}{\overset{\text{660-nm light}}{\rightleftharpoons}} \text{Pfr (active form)} \rightarrow \text{biological responses}$$

To understand the molecular basis of the physiological activity of the Pfr[1] form of phytochrome, structural and conformational differences between the two spectral forms have been studied in several laboratories [for reviews, see Furuya (1983) and Lagarias (1985)]. Most of the purified phytochromes used in these studies during the last two decades are now considered to have been proteolytically degraded phytochromes, consisting of either 60-kDa or 118/114-kDa monomers. It is now established that undegraded oat phytochrome has a monomeric molecular mass of about 124 kDa (Vierstra & Quail, 1982, 1983; Kerscher & Nowitzki, 1982; Hershey et al., 1985).

Significant differences between 124-kDa and degraded phytochrome molecules have been reported [for a review, see

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[1] Abbreviations: CD, circular dichroism; Pr, red-absorbing form of phytochrome; Pfr, far-red-absorbing form of phytochrome; kDa, kilodalton(s); HA, hydroxyapatite; KPB, potassium phosphate buffer; NaPB, sodium phosphate buffer; EDTA, ethylenediaminetetraacetate sodium salt; SAR, specific absorbance ratio (A_{666}/A_{280}), with phytochrome in its red-absorbing form; TNM, tetranitromethane; HPLC, high-performance liquid chromatography.

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Table I: Monoclonal Antibodies Used in This Study

antibody, monoclonal	epitope location	characterization (ref)
oat-13	chromophore domain away from N-terminus	Cordonnier et al. (1984, 1985)
oat-22	similar to oat-13 but does not compete for same site	Cordonnier et al. (1984, 1985), Shimazaki et al. (1986)
oat-25	N-terminus domain	Cordonnier et al. (1985), Shimazaki et al. (1986)
oat-26	C-terminus domain (nonchromophoric domain)	previously unpublished
oat-28	similar to oat-26 but does not compete for same site	previously unpublished
oat-31	undetermined	previously unpublished

Quail et al. (1983) and Lagarias (1985)]. For example, a photoreversible CD change in the UV region is observable only for 124-kDa oat phytochrome but not for degraded phytochrome preparations. This difference indicates that the photoreversible change in secondary structure of 124-kDa phytochrome requires the presence of the N-terminus domain that is absent in degraded phytochrome (Vierstra et al., 1987). To verify this suggestion and elucidate further the nature of interactions between the chromophore and its apoprotein, we report here the effects of monoclonal antibodies on the photoreversible CD change observed with 124-kDa oat phytochrome. The effect of chemical modifications of the chromophore on the photoreversible CD change has also been examined.

Materials and Methods

Phytochrome Purification. Undegraded, 124-kDa phytochrome was purified as previously described (Chai & Song, 1986). Briefly, phytochrome was partially purified from extracts of 4-day-old etiolated oat seedlings by ammonium sulfate precipitation and hydroxyapatite (HA) chromatography. The HA-purified phytochrome was then subjected to ammonium sulfate back-extraction. A supernatant fraction (II) was applied to a Bio-Gel A-1.5m column, which was previously equilibrated with 20 mM KPB, pH 7.8 (4 °C), containing 1 mM EDTA. Phytochrome was eluted from the column with the same buffer. Phytochrome with SAR values of 0.98–1.13 was routinely obtained with this procedure (Chai et al., 1987). All experiments reported here were performed with phytochrome of SAR = 1.13 and under green safety light at 4 °C, unless otherwise noted.

Phytochrome was photoconverted by irradiating with a Bausch and Lomb microscope illuminator combined with a 666-nm interference filter (Oriel C572-6600) for red light, and with a far-red cutoff filter (Ealing 26-4457) for far-red irradiation. Fluence rates were 7.5 W/m^2 and 1.6 kW/m^2, respectively.

Monoclonal Antibodies. Six monoclonal antibodies to 124-kDa oat phytochrome, which are designated oat-13, oat-22, oat-25, oat-26, oat-28, and oat-31, were obtained as described previously (Cordonnier et al., 1983, 1984, 1985). They were all immunopurified from spent hybridoma medium with a column of immobilized rabbit antibodies to mouse immunoglobulins (Cordonnier et al., 1983). These monoclonal antibodies have epitope characteristics as shown in Table I.

Circular Dichroism. CD spectra of phytochrome were recorded on a modified JASCO-20 CD/ORD spectropolarimeter equipped with a Morvue photoelastic modulator (PEM-3) and a lock-in amplifier (Ithaco Model 3941), instead of a Pockels cell, to increase the signal-to-noise ratio (Jung et al., 1980).

The CD instrument was calibrated with *cis*-androsterone (Kirk & Klyne, 1976). Reproducibility of results was regularly checked with standard substances, such as known proteins (bovine serum albumin; Barnes et al., 1972), as well as by repeating runs on several replicates of phytochrome solutions (without added antibodies or chemicals).

Measurements in the far-UV (200–250 nm) region employed cells of 2-mm path length and phytochrome monomer concentrations of about 0.6–1.6 μM. The CD spectra of each given phytochrome solution (or a mixture of phytochrome and monoclonal antibody) were obtained from an average of two or more consecutive scans. The CD spectrum of phytochrome (Pr or Pfr) was scanned first, and each sample was then photoconverted to the other form for a second CD scan. The CD spectra were recorded at least in duplicate and frequently in triplicate with independent phytochrome sample preparations. In all recordings, the CD spectra were reproducible.

Mean residue ellipticity was calculated from

$$[\theta]_\lambda = -[33(M/100)(A_L - A_R)]/lc$$

where $[\theta]_\lambda$ = decimolar ellipticity at a given wavelength, M = the mean residue weight, $A_L - A_R$ = the observed difference in absorbance of left and right circularly polarized light, l = the path length in decimeters and c = the concentration of protein in grams per milliliter. Concentration of phytochrome was calculated from an extinction coefficient of 1.32×10^5 M^{-1} cm^{-1} (Lagarias et al., 1986). The mean residue weight of 110.7 was calculated from a molecular weight of 124 870 on the basis of the known sequence of phytochrome (Hershey et al., 1985). Estimates of apparent α-helix, β-pleated sheet, β-turn, and random coil were computed by the method of Chang et al. (1978; Yang et al., 1986), which is derived from linear, least-squares analyses of the reference CD spectra of 15 proteins of known X-ray crystallographic structure. The computer program for the CD analysis of protein conformation was provided by Dr. C.-S. C. Wu and Dr. J. T. Yang, University of California, San Francisco.

For CD measurements, 200 μL of a monoclonal antibody [in 0.1 M NaPB containing 1 mM EDTA and 0.02% NaN_3 at pH 7.8 (4 °C)] was mixed with 200 μL of 20 mM KPB containing 1 mM EDTA, pH 7.8 (4 °C), to make a final concentration of 0.95 mg/mL. Two hundred microliters of each monoclonal antibody was also mixed with 200 μL of a 0.16 mg/mL solution of Pr or Pfr. At these concentrations, virtually all phytochrome in solution is complexed with antibody, since there is an approximately 8:1 molar ratio of antigen binding sites to phytochrome monomer. The far-UV CD spectra of monoclonal antibody and its mixture with 124-kDa phytochrome were recorded in a 2-mm path-length cell at room temperature. The short-wavelength CD spectra ($\lambda < 205$ nm) were recorded with diluted solutions when the photomultiplier voltage saturated. Solution stability and/or aggregation of phytochrome–monoclonal antibody mixtures was monitored by CD and by absorbance at 270 nm.

Tetranitromethane Oxidation and Borohydride Reduction. Absorbance measurements were carried out with a Lambda 3B (Perkin-Elmer) or HP 8451A (Hewlett-Packard) spectrophotometer. A stock solution of 2 mM TNM was prepared in ethanol (99.99%) (Hunt & Pratt, 1981). Samples for CD measurements were incubated for various times after addition of 5 μL of 2 mM TNM to 495 μL of Pr or Pfr forms of 124-kDa phytochrome (final concentrations of 40 μM TNM and 0.1 mg/mL phytochrome) at room temperature. CD measurements were carried out as a function of absorbance bleaching (i.e., 50% of original absorbance at 666 nm for Pr,

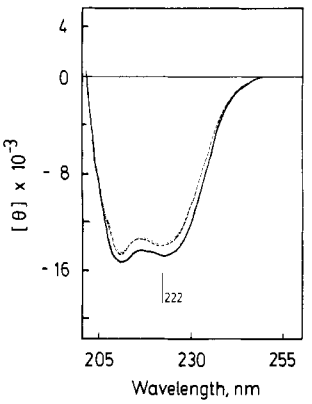

FIGURE 1: Far-UV CD spectra of 124-kDa phytochrome, which was prepared by the modified protocol (Chai & Song, 1986), in 20 mM KPB, pH 7.8 (4 °C), containing 1 mM EDTA. Spectra were obtained with a 2-mm path-length cell at room temperature. Mean residue ellipticity is expressed (on the basis of a mean residue weight of 110.7) in units of degrees centimeter squared per decimole. The final concentration of phytochrome was 0.95 mg/mL. (---) Pr; (—) Pfr; (···) cycled Pr.

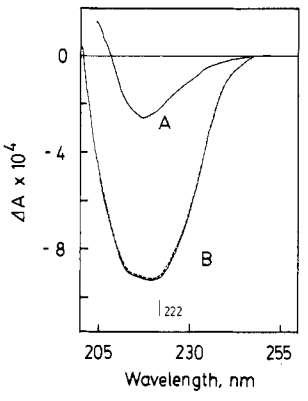

FIGURE 2: Far-UV spectra of monoclonal antibody oat-25 and its mixture with 124-kDa phytochrome (Pr or Pfr form) were obtained with a 2-mm path-length cell at room temperature. (A) 200 μL of oat-25 [0.95 mg/mL, in 0.1 M NaPB, pH 7.8 (4 °C), containing 1 mM EDTA and 0.02% NaN_3] was mixed with 200 μL of 20 mM KPB, pH 7.8 (4 °C), containing 1 mM EDTA. (B) 200 μL of oat-25 (0.95 mg/mL) was added to 200 μL of the Pr form of 124-kDa phytochrome (0.19 mg/mL) in 20 mM KPB, pH 7.8 (4 °C), containing 1 mM EDTA. (---) Pr; (—) Pfr; (···) cycled Pr.

or 730 nm for Pfr). Steady-state absorbance measurements were performed with a Lambda 3B spectrophotometer at 4 °C. Samples for each steady-state measurement were incubated for various times after addition of 5 μL of a 1 M solution of $NaBH_4$ in 20 mM KPB, pH, 7.8 (4 °C), to 495 μL of phytochrome (Pr or Pfr form, final concentrations of 20 mM $NaBH_4$ and 0.1 mg/mL phytochrome). After partial bleaching, CD measurements were obtained with the mixture at room temperature.

Spectral Bleaching Kinetics. Chromophore oxidation of phytochrome with TNM and chromophore reduction with sodium borohydride were followed spectrophotometrically at 660 nm (for Pr) and at 730 nm (for Pfr), immediately following addition of freshly prepared oxidant or reductant, in the same buffer as for CD measurements.

RESULTS

CD spectra of 124-kDa, etiolated oat phytochrome in its Pr and Pfr forms were obtained (Figure 1). A clear increase in ellipticity upon Pr-to-Pfr phototransformation is seen. Photoconversion of Pfr back to Pr (photocycled) produces a CD spectrum that is virtually identical with the spectrum of the original Pr form. This photoreversible CD change can be

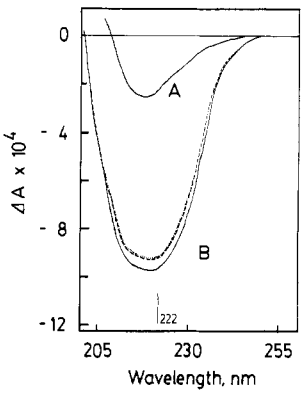

FIGURE 3: Far-UV CD spectra of monoclonal antibody oat-22 and its mixture with 124-kDa phytochrome were recorded with the same conditions as in Figure 2. (A) Monoclonal antibody oat-22 and (B) its mixture with 124-kDa phytochrome: (---) Pfr; (—) Pr; (···) cycled Pr.

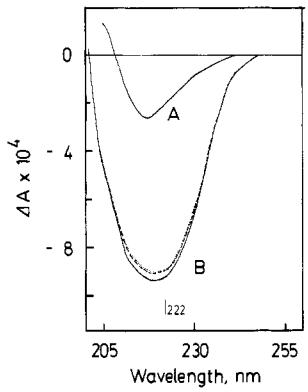

FIGURE 4: Far-UV CD spectra of monoclonal antibody oat-28 (or oat-26 which showed the same CD spectra) and its mixture with 124-kDa phytochrome were recorded under the same conditions as in Figure 2. (A) Monoclonal antibody oat-28 and (B) its mixture with 124-kDa phytochrome; (---) Pfr; (—) Pr; (···) cycled Pr.

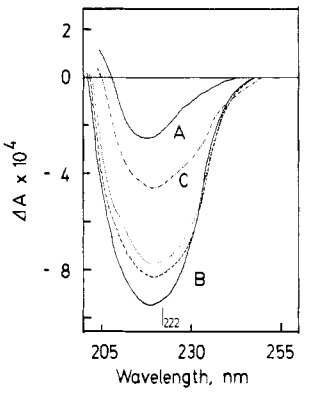

FIGURE 5: Far-UV CD spectra of monoclonal antibody oat-31 (or oat-13 which showed similar spectral behaviors) and its mixture with 124-kDa phytochrome were recorded under the same conditions as in Figure 2. (A) Monoclonal antibody oat-31 and (B) its mixture with 124-kDa phytochrome; (—) Pr; (---) Pfr; (···) cycled Pr. (C) After 2-h incubation of the mixture.

repeated on the same sample several times with successive red/far-red irradiation cycles. Results are consistent with previous observations (Vierstra et al., 1987).

The CD spectrum of phytochrome in its Pr form was obtained in the presence of oat-25, followed by successive CD recording after photoconverting the phytochrome to Pfr with red light, and then back to Pr with far-red light (Figure 2B). No photoreversible CD change was observed. For comparison, the CD spectrum of oat-25 alone is also shown (Figure 2A).

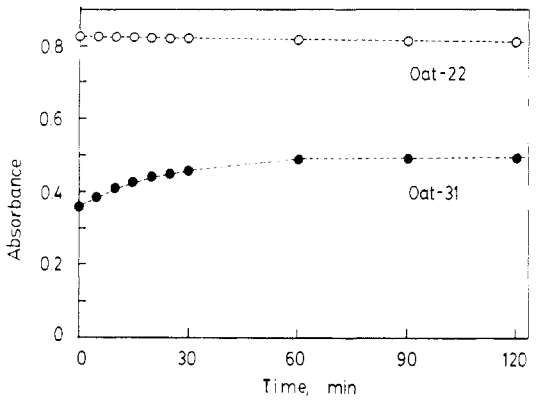

FIGURE 6: Absorbance changes with time at 280 nm for a mixture of 124-kDa phytochrome with monoclonal antibody oat-22 and with monoclonal antibody oat-31 under the same conditions as in Figure 2.

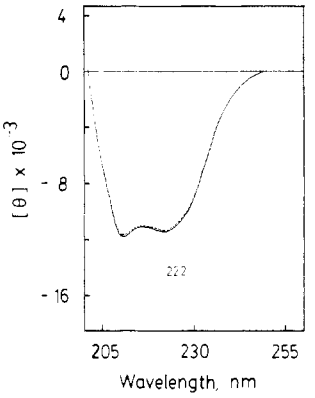

FIGURE 7: Far-UV CD spectra of a mixture of 124-kDa phytochrome and TNM (final concentration 40 μM) were recorded with a 2-mm path-length cell at room temperature after 50% bleaching (at 666 nm for Pr and at 730 nm for Pfr) at room temperature. 5 μL of 2 mM TNM in ethanol was mixed with 495 μL of phytochrome (final concentrations of 40 μM TNM and 0.1 mg/mL phytochrome) in 20 mM KPB, pH 7.8 (4 °C), containing 1 mM EDTA. (---) Pr and (—) Pfr.

When Pfr was mixed with oat-25, its CD spectrum was identical with that shown in Figure 2. No photoreversible CD change was observable when the Pfr–antibody mixture was photoreverted back to Pr, nor when it was cycled back to Pfr (spectra not shown). The CD spectrum of the phytochrome–oat-25 mixture is a sum of the independent CD spectra of phytochrome (Pr form) and oat-25.

Oat-22 (Figure 3), oat-26, and oat-28 (Figure 4) did not suppress the photoreversible CD change. However, oat-26 and oat-28 do appear to inhibit slightly the photoreversible CD change (compare the CD changes in Figure 4 with those in Figures 1 and 3).

The CD spectrum of phytochrome (Pr form) together with oat-31 is markedly diminished in intensity upon photoreversion to Pfr (Figure 5). Photocycling and further incubation of the photocycled phytochrome (Pr form) drastically reduced the CD intensity over the entire far-UV region (Figure 5). These same CD spectral patterns were observable starting with phytochrome in its Pfr form. Thus, both the Pr and Pfr species, cycled or not, substantially lost their CD intensities during incubation. Accompanying the gradual loss of CD intensity, the absorbance at 280 nm also increased (Figure 6), and phytochrome–antibody precipitates could be seen visually. Similar aggregation phenomena were observed with phytochrome–oat-13 mixtures (data not shown). All other phytochrome–antibody mixtures examined showed no apparent aggregation during incubation, as monitored by CD and by the absorbance at 280 nm (for example, see Figure 6).

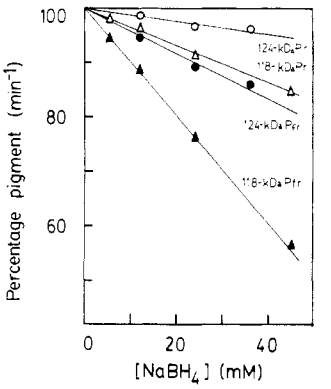

FIGURE 8: Borohydride reduction of Pr and Pfr at room temperature, pH 7.8 (4 °C), in 0.1 M NaPB [open and closed triangles for degraded 118/114-kDa phytochrome, taken from Song (1983b)] and in 20 mM KPB, pH 7.8 (4 °C), containing 1 mM EDTA (open and closed circles for 124-kDa phytochrome). The ordinate represents initial rates of reduction; the percentage pigment remaining was calculated after 1 min of reduction.

Table II: Analysis of the Secondary Structure of Phytochrome and Monoclonal Antibody Oat-25 according to the Method of Chang et al. (1978)[a]

protein	α-helix (%)	β-sheet (%)	β-turn (%)	random coil (%)
Pr[b]	52.2	0.0	21.1	26.7
Pfr[b]	55.3	0.0	18.8	26.0
oat-25[b]	0.0	15.0	36.5	48.5
Pr–TNM[c]	44.0	0	27.0	29.0
Pfr–TNM[c]	44.0	0	27.0	29.0
Pr–BH_4[d]	37.0	0.0	28.0	35.0
Pfr–BH_4[d]	38.0	0.0	26.0	36.0

[a] Ellipticity data were taken at 1-nm intervals in the range of 205–240 nm, unless specified otherwise. [b] Analysis of the CD spectra with data taken in the range of 205–240 nm. For four CD spectra analyzed, %α ± 1.15 (SD) and 2.53, %β, ± 0.65 and 0.96, and %RC ± 0.84 and 2.12, for Pr and Pfr, respectively. [c] Analysis of the spectra in Figure 7. Data taken in the range of 208–240 nm. [d] Analysis of the spectra in Figure 9. Data taken in the range of 208–240 nm.

To ascertain the role of the tetrapyrrole chromophore in the photoreversible CD change accompanying the phototransformation of phytochrome, we have also modified the chromophores of the Pr and Pfr forms of phytochrome with TNM and sodium borohydride. TNM preferentially oxidizes the chromophore, relative to tyrosyl residues (Hahn et al., 1984). TNM abolished the photoreversible CD change after a 50% bleaching of either Pr or Pfr (Figure 7). Borohydride reduces the tetrapyrrole chromophore preferentially (Scheer, 1984). Figure 8 shows spectral bleaching curves for the Pr and Pfr species of degraded (118/114-kDa) and 124-kDa phytochromes as a function of borohydride concentration and indicates that the Pfr chromophore is more susceptible to bleaching by reduction than is the Pr chromophore for both molecular mass species of phytochrome. The preferential accessibility of the Pfr chromophore relative to the Pr chromophore has also been observed with TNM (Hahn et al., 1984). Borohydride inhibits the extent of the CD change after a 50% bleaching of either Pr or Pfr (compare Figure 9 with Figure 1).

Discussion

Analysis of the secondary structure of phytochrome by the method of Chang et al. (1978) reveals that the Pr → Pfr phototransformation is accompanied by a 3% increase in α-helical conformation (Table II), in agreement with a previous estimate based on the simpler Greenfield and Fasman analysis

(1969) (Vierstra et al., 1987). The photoreversible CD change is not attributable to an aggregation effect, since the CD spectra (Figure 1) show no evidence of absorbance and CD flattening (Duysens' effect). Litts et al. (1983) were not able to detect any photoreversible far-UV CD change with their 124-kDa oat phytochrome. This discrepancy may be due to the different isolation and purification conditions used, as well as instrumental sensitivity differences. No β-sheet conformations were found in the present analysis.[2] According to method of analysis based on a linear combination of the CD spectra of 15 proteins with secondary structure known from X-ray crystallography (Provencher & Glöckner, 1981), the same trend was found (computations performed by Prof. H. Scheer). That is, an increase in α-helix upon Pr → Pfr phototransformation and the absence of β-sheet conformations in both Pr and Pfr forms of phytochrome were detected.

Since degraded oat phytochromes with molecular masses of 60 and 114/118 kDa do not exhibit any photoreversible CD change accompanying the phytochrome phototransformation (Hopkins & Butler, 1970; Anderson et al., 1970; Tobin & Briggs, 1973; Song et al., 1979; Hunt & Pratt, 1981), the observed photoreversible CD change in Figure 1 reflects a possible involvement of the N-terminus sequences in the red-light-induced α-helical folding. It is known that a segment of the N-terminus domain is cleaved off during isolation and purification of degraded phytochrome (Vierstra & Quail, 1982, 1983). The fact that 124-kDa phytochrome together with monoclonal antibody oat-25, whose epitope includes a primary sequence near the amino terminus, abolishes the photoreversible CD change (Figure 2) is consistent with the proposed α-helical folding of the N-terminus sequences in the Pfr form (Vierstra et al., 1987). This proposed α-helical folding may result from a possible interaction between the chromophore and apoprotein, including the amino-terminus segment (Song, 1983a, 1985; Vierstra et al., 1984; Jones et al., 1985). Thus, 124-kDa phytochrome bound by oat-25 behaves circular dichroically like degraded phytochrome that lacks the N-terminus segment. Moreover, Pfr bound by oat-25 reverts to Pr in the dark (Cordonnier et al., 1985), similarly to the dark reversion of degraded phytochromes. Pfr bound by oat-25 also exhibits a shorter wavelength absorbance maximum (Cordonnier et al., 1985), as is again the case for proteolytically degraded phytochromes that have lost their N-terminus. Thus, the N-terminus 6/10-kDa peptide segment is necessary to maintain proper chromophore–apoprotein interactions in the 124-kDa phytochrome (Jones et al., 1985).

Other monoclonal antibodies whose epitopes are removed from the N-terminus segment do not abolish the photoreversible CD change (Figures 3 and 4). Oat-28 and oat-26, however, do seem to reduce the magnitude of the CD change somewhat (Figure 4), suggesting that the nonchromophoric, C-terminus may be involved in the photoreversible CD change, perhaps cooperatively with the N-terminus segment. Because of a strong tendency to produce aggregates, effects of oat-13 and oat-31 on the CD spectra of phytochrome (Figure 5) cannot readily be analyzed in terms of secondary structural changes.

To ascertain the role of the phytochrome chromophore (especially Pfr) in α-helical folding of apoprotein, presumably

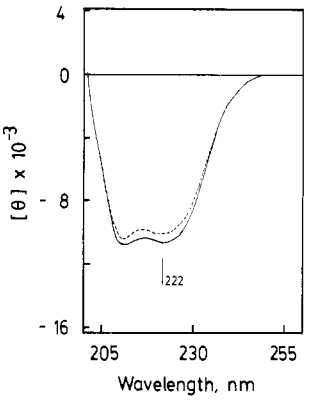

FIGURE 9: Far-UV CD spectra of the mixture of 124-kDa phytochrome (final concentrations of 20 mM $NaBH_4$ and 0.1 mg/mL phytochrome) and $NaBH_4$ (20 mM) were recorded with a 2-mm path-length cell at room temperature, after 50% bleaching of phytochrome (at 666 nm for Pr and at 730 nm for Pfr). (---) Pr; (—) Pfr.

the N-terminus segment, we have also examined the effects of TNM and borohydride. As summarized in Figures 7 and 9 and Table II, chromophore modifications interfere with the red-light-induced α-helical folding in the Pfr form of phytochrome. Since a 50% bleaching of the absorbances at 666 and 730 nm of the Pr and Pfr forms, respectively, almost completely abolishes the photoreversible CD change, TNM may have reacted with apoprotein before it oxidized the chromophores, thus reducing the mean residue ellipticities (Table II) and suppressing the photoreversible CD change. This possibility is reinforced by the observation that only 25% bleaching of the absorbances of phytochrome with 40 μM TNM was sufficient to yield the same CD as shown with 50% bleaching (Figure 7). With 25% bleaching by 20 μM TNM, approximately one-third of the photoreversible CD change was retained. Since tyrosyl residues react much slower with TNM compared to tetrapyrrolic chromophores (Hahn et al., 1984; Thümmler et al., 1985), it seems unlikely that the above results are explicable in terms of tyrosyl modifications. It is therefore likely that excess TNM oxidized cysteinyl residue(s) prior to the oxidation of the chromophores. It is well-known that TNM does react with cysteinyl residues readily (Hsieh & Matthews, 1981). However, the absorption spectra of phytochrome are not markedly affected by chemical modification of cysteinyl residues (Hunt & Pratt, 1981). Apparently, gross molecular integrity, including the dimeric quaternary structure of phytochrome, is preserved even after TNM oxidation, according to a HPLC analysis (Kwon et al., unpublished data).

Results of borohydride reduction of phytochrome are less complicated than those of TNM oxidation. A 50% bleaching of the phytochrome chromophore by borohydride results in only a 1% increase in α-helicity upon Pr → Pfr phototransformation (Figure 9 and Table II). Both TNM and borohydride modifications, however, lead to 20–25% loss of mean residue ellipticity, suggesting that the chromophore of phytochrome plays an important role, in addition to the N-terminus segment, in stabilizing the α-helical structure of the apoprotein. It is well-known that prosthetic groups such as the tetrapyrrolic heme of myoglobin (Beychok, 1966) tend to stabilize an α-helical conformation.

There are several interpretations of the data presented here. An attractive model, though simplistic, is that the photoreversible CD change accompanying the Pr → Pfr phototransformation reflects an enhanced α-helical folding of the N-terminus segment via a light-induced interaction between the peptide and the exposed Pfr chromophore. This interpretation is consistent with the results obtained here with monoclonal

[2] All previous estimations (Hopkins & Butler, 1970; Anderson et al., 1970; Tobin & Briggs, 1973; Hunt & Pratt, 1981; Vierstra et al., 1987) based on the Greenfield–Fasman analysis yielded somewhat less α-helix content than the results from the Chang et al. (1978) analysis (Table II). Furthermore, previous calculations yielded a significant amount of β-sheet structure. However, this is understandable since the Greenfield–Fasman analysis does not distinguish between β-sheet and β-turns.

antibodies, particularly oat-25, and with chromophore modifications. It is also consistent with the earlier observation that oat-25 exhibits about 5-fold greater activity with Pr than with Pfr in an enzyme-linked immunosorbent assay, which could be interpreted as an indication that the N-terminal domain of phytochrome undergoes a conformational change (Cordonnier et al., 1985). The N-terminus segment contains amino acid sequences (residues 16–36 and 44–52) that are helix-compatible according to the Chou–Fasman prediction (Chou & Fasman, 1978) using the MSEQ protein analysis program (Black & Glorioso, 1985). It may be merely coincidental, but it is interesting that these 28 amino acid residues are about 3% of the total amino acid residues of 124-kDa oat phytochrome (Hershey et al., 1985).

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