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Plant Light Signaling Mediated by Phytochromes and Plant Biotechnology

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Abstract

The plant pigment proteins phytochromes are a molecular light sensor or switch for photomorphogenesis involving a variety of growth and developmental responses of plants to red and far-red wavelength light. Underscoring the photomorphogenesis mediated by phytochromes is the light signal transduction at molecular and cellular levels. For example, a number of genes activated by the phytochrome-mediated signal transduction cascade have been identified and characterized, especially in Arabidopsis thaliana. The light sensor/switch function of phytochromes are based on photochromism of the covalently linked tetrapyrrole chromophore between the two photoreversible forms, Pr and Pfr. The photochromism of phytochromes involves photoisomerization of the tetrapyrrole chromophore. The "photosensor" Pr-form ("switch off" conformation) of phytochromes strongly absorbs 660 nm red light, whereas the "switch on" Pfr-conformation preferentially absorbs 730 nm far-red light. The latter is generally considered to be responsible for eliciting transduction cascades of the red light signal for various responses of plants to red light including positive or negative expression of light-responsive genes in plant nuclei and chloroplasts. In this paper, we discuss the structure-function of phytochromes in plant growth and development, with a few examples of biotechnological implications.

INTRODUCTION

UV and visible wavelength light are environmental signals for plant growth and development. The chemical and structural identities of the photoreceptors are not fully known. Flavins are generally accepted as likely chromophores for blue light receptors. Phytochromes are the best characterized photoreceptors for plants (for review, see Kendrick & Kronenberg, 1994). Only a few chromophores have been chemically identified for various photobiological receptors including retinal, phytochromobilin, flavins, p-coumaric acid thioester, stentorin and blepharismin; the last three being the latest additions to the exclusive list of the photoreceptor chromophores (Tao et al., 1993;Checcucci et al., 1997; Hoff et al., 1994; Baca et al., 1994). From the list of photoreceptor

chromophores, only two, flavin and tetrapyrrole, have been identified for higher plant photobiology. The exact chemical identities of the flavin blue light receptors have not yet been fully established.

The molecular basis of the photo-signal transduction is the reversible photochromic change between the Pr and Pfr forms of the light switch molecule.

red light
Pr ========== Pfr -------- Biological actions
far red light

Recent studies show that the scheme of phytochrome action appearing in most textbooks and reviews prior to 1993 is a gross over-simplification (Shinomura et al., 1994, 1996; Quail et al., 1995). For example, the reversible photochromic response expected from Pfr photoreversion does not apply to phytochrome A (phyA) in vivo, although it is readily demonstrable in solution. This paper will discuss primarily the structure and function of phyA.

PHYTOCHROMES AS LIGHT SENSORS AND SWITCHES

Phytochromes as UV photosensors.

Phytochromes are generally not regarded as UV photoreceptors, although they absorb strongly in the UV region. The purest oat phyA we were able to obtain had a specific absorbance ratio [A at 280 nm:A at 660 nm for Pr] of 1.10 or greater. The chromophore absorbance at 280 nm in native oat phyA is estimated to be 19,700 M-1cm-1, assuming no hypochromism of the 10 Trp and 21 Tyr protein absorption. The phytochromobilin chromophore therefore accounts for slightly less than 17 % of the total molar absorbance at 280 nm. Synthetic phytochromobilin-peptide absorbs only moderately at 280 nm (e280 14,000 M-1cm-1; Micura and Grubmayr, 1995). Molar UV extinction coefficients for native Pr- and Pfr-phytochromes A are essentially identical, so irradiation of phyA, for example at 280 nm, should yield a 50:50 Pr/Pfr photoequilibrium mixture. Any significant deviation from this value may represent differential contributions of the Trp residues as energy donors to the chromophore within the native phytochrome molecule. The phototransformation of phytochrome at 301 nm is primarily caused by absorption by the chromophore. We calculated rate constants for the UV phototransformation of 124-kDa full-length and 114/118-kDa degraded phytochromes for phototransformation with 301 and 276 nm excitations. The lower %[Pfr] in the photoequilibrium mixture of degraded phytochrome is caused in part by dark reversion, which is negligible for 124-kDa phytochrome under the experimental conditions used.

The photostationary %[Pfr] levels achieved by UV excitation are near theoretical values (68-69%; Lagarias et al., 1987) and are independent of the fluence rates used for 124-kDa phytochrome. A slightly higher value of the Pfr level under 276 nm, compared to that obtained under 301 nm excitation, may reflect energy transfer from the Trp residues to the chromophore. Thus, UV signals can be perceived by phytochromes and may elicit phytochrome-mediated responses in plants, through photoreversible transformation either by direct excitation of the chromophore or via energy transfer from Trp residue(s) to the chromophore.

Phytochromes as near UV blue light sensors.

The absorption spectra of phytochromes show a bathochromic shift of the near-UV absorbance maximum at 379-381 nm in Pr to 402-404 nm in Pfr. Although the near-UV to blue spectral shift is only one-third of the red/far-red photochromic shift in wavelength, it represents a similar magnitude of shift in energy (1,440 cm-1 to1,320 cm-1). At photoequilibrium, monochromatic excitation of 124-kDa oat phyA

at 380 nm and 404 nm yielded 47.0 and 43.5 %[Pfr] respectively. These values are approximately within the range predicted from the absorbance spectra (Lagarias et al., 1987). The near-UV/blue spectral shift in wavelength is considerably smaller than the red/far-red photochromism and the %[Pfr] level does not depend sharply on wavelength in this spectral region, so it is difficult to demonstrate direct involvement of phytochromes in blue light-mediated responses in plants. However, lack of near-UV/blue reversibility does not rule out a direct role for phytochrome in blue light phenomena.

Co-action of blue light receptor systems with phytochromes in vivo is well known (for review, see Mohr, 1994). The molecular mechanism(s) for the co-action remains obscure. However, direct near-UV/blue excitation of phytochrome may not play a significant role in the co-action phenomena. The phytochrome system might be activated by blue light in a non-additive way beyond the level of %[Pfr] achievable with direct near-UV/blue excitation. For example, flavins bound or localized at/near the active phytochrome molecules/complexes can transfer near-UV and blue wavelength excitation energy to the latter, in a manner similar to the action of photosynthetic antenna systems and the sensitized luminescence of rare-earth ions excited by Trp residues in certain calcium binding proteins. The critical energy transfer distance between flavin and phytochrome ranges from 26 angstrom for Pr-phytochrome to 22 angstrom for Pfr-phytochrome. Energy transfer may also be specific. For example, flavin (FMN) preferentially photosensitizes the forward phototransformation of phytochrome from Pr to Pfr in solution (Sarkar and Song, 1982). As in photosynthetic antenna systems, carotenoids can transfer their excitation energy to phytochromes, but this has not been demonstrated either in vivo or in vitro. Perhaps phytochrome-mediated action spectra can be matched with flavins and/or carotenoids if the population of blue light absorbers far exceeds that of phytochrome in its functional localization site. No physiological experiments to test the contributions of these energy transfer routes to the crptochrome-phytochrome co-action phenomena have been reported.

Photochromic light sensors/switches. Using truncation and deletion mutants of pea apophytochromes A (Deforce et al., 1991, 1993; Bhoo et al., 1997), we studied the chromophore ligation and the chromophore-apophytochrome interactions. Autocatalytic reconstitution of pea phyA from the free chromophore and apophytochrome did not require the presence of the first 45 amino acids (Deforce et al., 1991). However, a deletion mutant lacking 222 amino acid residues from the N-terminus failed to reconstitute holophytochrome. Cherry et al. (1992, 1993) refined this result, demonstrating that resides between the amino acid residues 70 and 398 are required for the autocatalytic reconstitution of phytochrome.

To further probe the specific interactions between the chromophore and the apoprotein, the effects of single amino acid substitutions of conserved residues in the vicinity of Cys323 were investigated (Deforce et al., 1993). We found all ten site-specific mutants specifically assembled the chromophore with varying efficiencies. The Cys323 thiol group in these mutants can attack the ethylidene double bond of ring A, yielding a covalent linkage between the apoprotein and the chromophore.

Beyond the chromophore site, isoleucine-80 and the buried a-helical motif along the residues 360-400 appear to play critical roles for the chromophore-apophytochrome interactions involved in the photochromism of phytochrome A (Bhoo et al., 1997). Other peptide segments within the N-terminal domain of first 600 amino acid residues seem to play only a minor role in affecting the spectral maxima of the Pr and Pfr forms of phytochrome A.

Based on the results obtained so far, we can

describe the minimal photochromism-conferring interaction between the tetrapyrrole chromophore and the apophytochrome peptide chains in terms of acid/base or hydrogen-bonding function of His residue, the amphiphilic a-helical crevice for the chromophore, and hydrophobicity of the chromophore binding site. Relative catalytic and/or thermodynamic roles of these interactions in chromophore ligation, photoisomerization and the stabilization (and thus absorbance maxima) of the Pr and Pfr chromophore conformations remains to be elucidated.

Inter-domain signalling in the phytochrome molecules. To address the question about the possible interaction between the N-terminal chromophore-containing and C-terminal dimerizing domains in pea phytochrome A, seven carboxyl-terminal deletion mutants and wild type of pea PHYA were expressed in Saccharomyces cerevisiae. The mutant clones were amplified and the identities of the newly generated mutant PHYA open reading frames were verified by restriction mapping and sequencing. A series of seven carboxyl-terminal deletion mutants of PHYA were successfully produced. They are PHYAD248-1124, PHYAD94-1 24, PHYAD601-1124, PHYAD653-1124, PHYAD731-1124, PHYAD772-1124, and PHYAD1050-1124, designated as M1 through M7. Western blot analysis with the monoclonal antibodies mAP13 and mAP5, specific for pea PHYA, showed that the transgenic yeasts expressed truncated phytochrome apoproteins of the expected mass for each deletion: M1, 27 kDa: M2, 53 kDa; M3, 65 kDa; M4, 70 kDa; M5, 79 kDa; M6, 83 kDa; and M7, 113 kDa, and wild type pea PHYA, 121 kDa, respectively.

We observed that deletion of the C-terminus to residue 630 did not interfere with the rapid covalent linkage of PCB to the apoprotein and its photochromic effect. This is consistent with our previous finding that pea PHYAD549-1124 self-assembled the phytochrome-characteristic photochromism (Deforce et al., 1991). The reconstituted PCB-phytochrome species involve an autocatalytic covalent addition of the chromophore to the apoprotein. This can be detected by a Zn2+-induced fluorescent band on the gel, corresponding to the same position by Western blot analysis.

Except for the deletion mutant PHYD248-1124, wild-type/full-length PHYA and all other mutant PHYA's yielded red/far-red reversible photochromic products when incubated in the presence of PCB. Absorption maxima for the recombinant full-length PHYA were at 656 nm and 718 nm for the Pr and Pfr forms, respectively. These values are consistent with those reported by Deforce et al. (1993). Both absorption maxima are blue-shifted relative to those (666 nm and 729 nm, respectively) for native pea phytochrome A. Absorbance maxima for the deletion mutants are also blue-shifted compared with wild type recombinant and native phytochromes. This suggests that the C-terminal domain and the dimerization affect the chromophore domain, indicative of an inter-domain signaling within the phytochrome A molecule.

Several models have been proposed for the dimerization contacts between the C-terminal domains of the two monomer subunits. We proposed a phytochrome dimer model involving two anti-parallel b-sheet or a-helix segments localized between residues 730 and 821 (Romanowski & Song, 1992). Using a series of deletion mutants of oat phytochrome A, Edgerton and Jones (1993) have shown that residues 596-683 are directly involved in dimerization and that residues 685-815 interact with the 596-683 contact segments. We are currently examining both models by using deletion mutants.

The PHYAD248-1124 mutant missing 877 amino acid residues from the C-terminal end did not incorporate PCB, as determined by its lack of photochromism (M1 = PHYAD248-1124) or Zn2+-induced phycocyanobilin fluorescence. This is not surprising since this deletion mutant has

lost its Cys323 residue for the thioether linkage formation.

SIGNALING ROLE OF PHYTOCHROMES

Post-translational modification and signal transduction. The N-terminal Ser residue in oat phyA is N-acetylated. The role of N-acetylation in the structure and function of phyA is not known. It is an open question whether N- and O-glycosyl groups are present in phytochrome. phosphorylation is a likely post-translational modification reaction with implications in phytochrome-mediated signal transduction (Singh and Song, 1990). Phytochromes are cytosolic proteins; perhaps an early molecular response to a red light signal perceived by phytochrome molecules involves post-translational modifi - cations such as phosphorylation in the cytosolic phase.

Protein phosphorylation. Protein kinase/phosphatase cascades involving regulatory proteins are necessary to certain signal transduction processes in bacteria, yeast and animals (Ota & Varshavsky, 1993; Chang et al., 1993; review by Koshland, 1993). This appears to be true in plants also; phosphorylation/dephosphorylation of certain proteins has been shown to be modulated by red-/far red-light via phytochrome (Moessinger et al., 1984; Otto & Schaefer, 1988; Romero et al., 1991; for review, see Singh & Song, 1990; Roux, 1994). In fact, evidence that red light and phytochrome rapidly induce protein phosphorylation I plant protoplasts is increasing (Fallon et al., 1993, 1994; Harter et al., 1994). Datta and Cashmore (1989) showed that binding of the transcription factor AT-1 to the promoters of some phytochrome-mediated light responsive genes in pea is regulated by reversible phosphorylation. Phytochrome A in etiolated oat seedling extract also appears to be tyrosine phosphorylated, with red light eliciting its rapid dephosphorylation (Sommer & Song, 1996). In at least one phytochrome species from Ceratodon, the phytochrome molecule itself apparently contains a tyrosine kinase-motif (Thuemmler et al., 1992). Involvement of a protein kinase may occur early in the signal transduction pathway. A variety of plant protein kinases and their genes have been detected and cloned (Mizoguchi et al., 1994). As in animal cells, components for the MAP kinase cascade are present in plants. Park & Chae (1990) used oat protoplasts to show red light (and phytochrome by implication) modulation of PKC activity.

Do protein phosphorylation of phytochromes and its induced conformational change play a role in light signal transduction, paticularly via the inter-domain signaling between the photosensory N-terminal domain and the signal transducing regulatory motif of the C-terminal domain? Phosphorylation of a Ser residue near the N-terminus of glycogen phosphorylase in glycogen phosphorylase is noteworthy because it dramatically affects the local conformation (Barford et al., 1991). This change, triggered by Ser-phosphorylation, serves as a signal transducing element which induces tertiary and quaternary structural changes leading to allosteric activation of the enzyme (Johnson & Barford, 1993). Recently, Johnson & Barford (1993) reviewed the effects of phosphorylation on the structure and function of proteins. There have been surprisingly few CD studies on the effect of phosphorylation on protein conformations. We found two recent papers reporting phosphorylation effects on protein conformation (Ramwani et al., 1989; Wang & Creutz, 1994). Using CD analysis, Ramwani et al. (1989) found that a profound conformational change (b-sheet formation) could be induced by phosphorylation at a single specific site, but phosphorylation at other sites had no effect (so-called "silent phosphorylation"; Johnson & Barford, 1993). It is possible that the negative phosphate group contributes to the stabilization of a secondary structure via hydrogen

bonding and electrostatic forces (Stoner, 1984).

Oat phyA is phosphorylated in vitro at Ser17 and in vivo at Ser7 (conserved in other phyA species) in the amino terminal segment of Pr, and also at the hinge region Ser598 in Pfr (Wong et al., 1986; Lapko et al., 1997). Ser598 is not conserved in pea phyA, but Thr600 is conserved among dicot phytochromes. Thr595, in the hinge region, is another phosphorylation site in the Pfr form of phytochrome. If these phosphorylations are significant in phytochrome-mediated signal transduction, modulation of phytochrome "activity" by phosphorylation/dephosphorylation probably requires a conformational change in the phytochrome molecule. The implications of such conformational changes in signal transduction are far-reaching.

By using CD spectroscopy, we examined the conformations of Pr and Pfr before and after phosphorylation by a protein kinase and protein kinase A (Lapko et al., 1996). The results suggest that the phosphorylation of phyA at the N-terminal and hinge Ser residues is of the "silent phosphorylation" type. We are currently investigating whether or not the inter-domain signaling within the phyA molecules is modulated or switched on/off by the phosphorylation at N-terminus and/or at the hinge region.

"Non-silent" phosphorylation. To study phosphorylation-dependent conformational changes in phytochrome, we used protein kinase A (PKA). Wong et al. (1986) showed that PKA phosphorylates oat Pr-phytochrome (0.9 Pi/monomer) to a greater extent than Pfr-phytochrome (0.65 Pi/monomer). When 32P-phosphorylated phytochrome (0.9 mol phosphate per monomer) was mixed with untreated phytochrome and applied to SDS PAGE, a radioactive label became uniformly distributed among phytochrome bands. Phosphorylation and dephosphorylation (with alkaline phosphatase) did not influence the mobility of oat phytochrome under native gel electrophoresis conditions. However, the Pfr-form displayed slightly higher mobility.

Tryptic results showed that phytochrome phosphorylation induces subtle conformational changes in both Pr and Pfr forms of 124-kDa phytochrome. However, we did not observe any significant differences in tryptic digestion patterns between the Pr- and Pfr-phosphorylated phytochrome preparations. This was unexpected, because preferential phosphorylation sites for Pr and Pfr are different (McMichael and Lagarias, 1990).

To interpret our observed data, we examined the possibility of phytochrome (as Pr and Pfr) phosphorylation at a third site in the chromophore domain, as described by Wong et al. (1986). Limited proteinase V8 digestion of phytochromes also revealed the influence of Ser598- phosphorylation at the hinge region. V8 digestion of phosphorylated phytochromes produced chromophore-containing polypeptides at 63- and 69-kDa. These polypeptides were apparently the result of a 3-kDa increase to the corresponding chromophore-containing fragments at 60- and 66-kDa for unphosphorylated Pr-and Pfr-phytochromes, respectively. Immunoblotting of the 8 h-proteinase V8 digest with Oat-25 showed that formation of a 40-kDa chromophore fragment with an intact N-terminal chain resulting from hydrolysis at the Glu353-Gln354 peptide bond (Grimm et al., 1987) was significantly inhibited in Pr-phosphorylated phytochrome. To examine the possibility that phosphate removal from native unphosphorylated phytochrome (not treated with PKA) enhanced the formation of the 40-kDa fragment, we performed comparative analyses of the initial steps of digestion for untreated and alkaline phosphatase treated phytochromes. Dephosphorylation did not influence the formation of this fragment, evidence that the phosphate attachment site in untreated phytochrome is different from the PKA phosphorylation site (Ser17) in Pr-phytochrome.

Pfr-phytochromes are preferentially phosphorylated at Ser598 by PKA, whereas Pr-phytochromes are

phosphorylated at both the N-terminal Ser17 and hinge Ser598. The latter pattern is different from that reported by Wong et al. (1986). They found an additional phosphorylation site recognized by phytochrome associated kinase in the Ser-rich N-terminus of oat phytochrome.

Limited tryptic digest clearly showed that phosphorylation at the hinge region induced a noticeable effect on the chromoprotein conformation, especially locally. The peptide bond Lys536-Asn537 was more exposed in phosphorylated phytochromes than in untreated/unphosphorylated chromoprotein. Limited tryptic digest of phosphorylated phytochromes inhibited hydrolysis at the Lys594-Arg-Glu596 peptide bonds and produced a significant amount of a 63-kDa fragment. Furthermore, phosphorylation caused peptide bonds near Ser598 to become more resistant not only to trypsin but also to digestion with V8 which recognizes negatively charged side chains (Glu and Asp). The Pr to Pfr phototransformation produces conformational changes in the vicinity of the phytochrome chromophore, a region rich in acidic residues. These changes in turn expose the Glu353-Gln354 peptide bond which is then readily cleaved by proteinase V8, producing a 40-kDa fragment with the intact N-terminus. We found this Pfr specific fragment at various time points during the digestion. Formation of this fragment was strongly inhibited in Pr-phosphorylated phytochrome. The phosphorylation site at Ser17 appears to be positioned near this peptide bond.

Tryptophan residues can be used as fluorescent probes for conformational changes produced by phototransformation of phyA. Interpretation of the data from dynamic fluorescence quenching studies is complicated by the presence of 10 Trp, but is somewhat simplified if fitting the tryptophan fluorescence decay is limited to two components, as described by Wells et al. (1994). In the present study these residues were exploited to determine if topographic alterations occur in response to phosphorylation at S17 and S598.

The cationic quencher Cs^+ was chosen because it can assess both the electrostatic and steric shielding of tryptophans and was best able to probe the collision cross sections of the Pr short component regardless of the state of phosphorylation. However, we observed an increase, approximately two fold, in the collision rate constant for both phyA phosphorylated as Pr and Pfr over untreated chromoprotein. Because this increase in kq occurred for both species it is probably a result of phosphate (a substrate for PKA catalysis in both photoisomers) incorporation at S598. Phosphorylation did not induce nearly as much change in the kq long component as in the short component. The value obtained for the red absorbing form of Pr phosphorylated phytochrome was larger than that for the far-red absorbing form, the reverse of results observed for untreated/unphosphorylated phyA. Chromoprotein phosphorylated as Pfr behaved similarly to that phosphorylated as Pr. We conclude that phosphorylation at Ser598 alters the microenvironments of some of the tryptophans and is a possible source of structural change as well as light driven chromophore isomerization.

Photochromic signaling. As mentioned in the previous section, the characteristic photochromism of phytochromes results from interaction between the phytochromobilin chromophore and the apophytochrome polypeptide, including the covalent linkage. Free phytochromobilin does not exhibit any significant photochromic shift of the red absorbance maximum (Qy band) upon irradiation, even though red irradiation isomerizes the chromophore (Bhoo et al., 1997). Covalent linkage of the chromophore to the apophytochrome via a Cys323-thioether bond is autocatalytic (Lagarias & Lagarias, 1989). We produced recombinant apophytochromes in yeast (Deforce et al., 1991, 1993; Bhoo et al., 1997) to study the chromophore ligation and the chromophore-

apophytochrome interactions. Various deletion-mutant studies showed that autocatalytic reconstitution of pea phyA from the free chromophore and apophytochrome can occur without the first 45 amino acids (Deforce et al., 1991). However, a deletion mutant lacking 222 amino acid residues from the N-terminus failed to reconstitute holophytochrome. Cherry et al. (1992, 1993) refined this result and demonstrated that the peptide segment between amino acid residues 70 and 398 is required for autocatalytic reconstitution of phytochrome. A mutant, comprising a deletion of the C-terminus to amino acid residue 548, in pea phyA correctly assembled red/far-red photoreversible phytochrome, indicating that the chromophore-apophytochrome interactions necessary for the red/far-red photoreversible transformation (photochromism) of phytochrome do not directly involve the entire C-terminal domain (Deforce et al., 1991). However, the C-terminal domain is important for dimerization and affects the absorbance maxima of the Pr and Pfr forms of phyA. This suggests that the C-terminal domain also interacts with the chromophore domain (Tomizawa et al., 1995; Park et al., 1996), although the model revealed from small angle X-ray scattering data does not show a direct contact (Nakasako et al., 1990).

Analysis of the site-specific mutagenesis data showed that all ten mutants, except possibly the His324 mutants, specifically assembled the chromophore with varying efficiencies, evidence that the substitutions did not cause all-or-none types of perturbations in the gross structure of apophytochrome. This indicates that the thiol group at position 323 in all pea phytochrome mutants can attack the ethylidene double bond of ring A, yielding a covalent linkage between the apoprotein and the chromophore. It seems reasonable that the residues substituted at Asp309, Arg318, His321, and Gln326 are conformationally/sterically rather than catalytically involved in the chromophore ligation. However, the His324 mutants were severely affected in both chromophore binding efficiency and photoreversibility of the reconstituted proteins. This residue is located immediately adjacent to the chromophore attachment site, so the inhibitory effect of the histidine substitutions is probably a result of either an altered positioning of Cys323 and the chromophore or an impaired catalytic role of the histidine residue for the chromophore ligation. A site-directed mutagenesis study indicates that the latter is the likely cause (Bhoo et al., 1997).

Ser322 in a phycocyanopeptide forms a seryliminoester with ring A, transforming it to the tautomeric structure as model for the Pfr chromophore (Micura and Grubmayr, 1995). However, a preliminary site-specific mutation (Ser Thr) study suggested that this residue is not critical for photochromism of phyA. Arg318 might electrostatically anchor the chromophore via one of the chromophore propionate side-chains, but direct electrostatic interaction between Gln326 and the ring B and C nitrogens of the chromophore seems unlikely. This residue is highly conserved among the known PhyA species, so it is puzzling that the effect of mutation at Gln326 on the autocatalytic ligation and photochromic properties of phytochrome was only modest (Parker et al., 1994).

Light-induced conformational changes. According to a topographic model for the phototransformation of phytochrome, the Pfr chromophore is more exposed than the Pr chromophore (Song, 1988; Hahn et al., 1984; Chai et al., 1987; Singh et al., 1989). However, the relative degree of exposure of the Pfr-chromophore depends on the N-terminal chain of the protein, because the 6-kDa peptide chain along the N-terminal sequences shields and interacts closely with the Pfr chromophore (Hahn et al., 1984). The marked effect of the N-terminal region on the Pfr spectrum (Vierstra & Quail, 1983) and differential proteolytic digestion patterns for Pr and Pfr

suggest that the N-terminal:chromophore interaction is specifically modulated by the phytochrome phototransformation.

The chromophore moves relative to the N-terminal chain and the distance between the chromophore and the N-terminus shortens upon the Pr Pfr phototransformation (Farrens et al., 1992). In addition to the decrease in N-terminal exposure upon conversion from Pr to Pfr, there is evidence that this region assumes an amphiphilic a-helical conformation in the Pfr form. Further evidence for interactions between the chromophore and the N-terminal peptide chain comes from CD studies (Vierstra et al., 1987; Chai et al., 1987; Sommer & Song, 1990; Deforce et al., 1994). A photoreversible CD change occurs during the phototransformation of 124-kDa phytochrome, corresponding to a reproducible 3 - 5% increase in a-helical folding in the Pfr form of phytochrome. However, no such photoreversible CD changes have been observed with proteolytically truncated phytochromes lacking the ca. 6-kDa N-terminal chain (primarily 114/118-kDa species). Time-resolved UV-CD has shown that complete helical folding of the N-terminus occurs between 100 msec and 1 sec after red light (666 nm) irradiation (Chen et al., 1993).

The spectral properties of phytochrome (lmax 730-732 nm for 124-kDa Pfr-phytochrome, compared to 720 nm for the N-chain-truncated 118-kDa Pfr-phytochrome) can be accounted for by: (1) the chromophore interacts with or is partially shielded by the N-terminal chain (Hahn et al., 1984), (2) the N-terminal a-helix is amphiphilic (Parker and Song, 1990; Parker et al., 1992), and (3) tetrapyrrole/heme prosthetic groups induce a-helical folding [e.g., hemoglobin and myoglobin (Beychok, 1966), heme-amphiphilic peptides (Robertson et al., 1994), chlorophyll-binding proteins (Paulsen et al., 1993) and phytochrome (Singh and Song, 1989)]. We suggest that anti-parallel folding of the two N-terminal a-helices accommodates the partially exposed part (ring C and D) of the chromophore as it reorients during Pr Pfr phototransformation. Although the molecular mechanism of interaction between the Pfr-chromophore and the N-terminus sequence is not known, amino acid residues near the N-terminus include serine and threonine clusters (compare moss phytochrome; Thuemmler et al., 1992). It is possible that some of these hydroxyl amino acid residues form hydrogen bonds with the pyrrole nitrogens, carbonyl and carboxylate groups of the chromophore.

The N-terminal sequence of oat phyA also contains one highly negatively charged region (Asp28 - Asp53) with a central highly conserved segment (Glu36 - Ser45). The extreme N-terminal region, Arg4 -Arg20, is rich in basic residues, and also contains clusters of 10 Ser residues in both oat and pea phytochromes. These regions may well be involved in the interaction with the chromophore via H-bonding and electrostatic forces. Gly54 is flanked on the carboxyl side by a highly conserved region, Gln58 - Lys76, rich in basic residues. This region is proteolytically sensitive in both Pr and Pfr, whereas the N-terminal chain contains only the Pr-sensitive proteolytic site. Within the 54-amino acid chain, there are 8 pairs of charged residues in both oat and pea phyA spaced seven residues apart, roughly two turns of an a-helix. Micelle-induced helical folding of the 54-mer showed that this peptide possesses high amphiphilicity (Parker & Song, 1992; Wells, 1996).

Site-specific mutations at the peaks of the hydrophobic moment values could perturb or abolish the amphiphilicity of the N-terminal a-helix. Substituting all of these Ser residues with Ala actually enhanced the biological activity of phytochrome (Stockhaus et al., 1992). This may be caused by an increase in the amphiphilicity of the N-terminal chain of the Ser Ala mutant (Song et al., 1996). Several sections

of the N-terminal chain of phyA are capable of folding as amphiphathic helices (Parker and Song, 1990). Calculations on oat phyA were based on 11 residue segments. Residues 19-29 have a hydrophobic moment of 0.406. Residues 44-54 have a value of 0.428 and residues 64-74 have a value of 0.473. The pea phyA N-terminal sequence showed a similar hydrophobic moment profile. The magnitude of these moments for all three of these segments indicates the possibility of forming an amphiphathic helix.

BIOTECHNOLOGICAL IMPLICATIONS

Since phytochromes mediate a number of photomorphogenic responses of higher plants to visible wavelength light, one can anticipate a variety of biotechnological applications of the phytochrome structure and function. We mention here only a few possible applications: (1) It may be feasible to enhance the germination rate of plant seeds in total darkness if the phytochrome A molecule is site-specifically mutated to fix its conformation in the Pfr form. (2) Plants under canopy conditions exhibit shade-avoidance responses in search of red light. Since far-red wavelength light is still abundant in canopy, it should be possible to stimulate greening and increase the yield of vegetative and cereal plants by overexpressing phytochromes, especially phytochrome A as the far-red wavelength light sensor. (3) By either mutating phytochrome B or blocking its synthesis by the antisense gene, it may also be practical to induce early flowering in certain late blooming and day-length sensitive flowering plants. Some of these biotechnological applications of phytochromes are being pursued at Kumho Life & Environmental Science Laboratory (see KLESL Annual Reports).

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