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Resonance Raman Analysis of the P_r and P_{fr} Forms of Phytochrome[†]

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ABSTRACT: Resonance Raman vibrational spectra of the P_r and P_{fr} forms of oat phytochrome have been obtained at room temperature. When P_r is converted to P_{fr}, new bands appear in the C=C and C=N stretching region at 1622, 1599, and 1552 cm^{-1}, indicating that a major structural change of the chromophore has occurred. The P_r to P_{fr} conversion results in an 11 cm^{-1} lowering of the N–H rocking band from 1323 to 1312 cm^{-1}. Normal mode calculations correlate this frequency drop with a $Z \rightarrow E$ isomerization about the $C_{15}{=}C_{16}$ bond. A line at 803 cm^{-1} in P_r is replaced by an unusually intense mode at 814 cm^{-1} in P_{fr}. Calculations on model tetrapyrrole chromophores suggest that these low-wavenumber modes are hydrogen out-of-plane (HOOP) wagging vibrations of the bridging C_{15} methine hydrogen and that both the intensity and frequency of the C_{15} HOOP mode are sensitive to the geometry around the C_{14}–C_{15} and $C_{15}{=}C_{16}$ bonds. The large intensity of the 814-cm^{-1} mode in P_{fr} indicates that the chromophore is highly distorted from planarity around the C_{15} methine bridge. If the $P_r \rightarrow P_{fr}$ conversion does involve a $C_{15}{=}C_{16}$ $Z \rightarrow E$ isomerization, then the intensity of the C_{15} HOOP mode in P_{fr} argues that the chromophore has an *E*,anti conformation. On the basis of a comparison with the vibrational calculations, the low frequency (803 cm^{-1}) and the reduced intensity of the C_{15} HOOP mode in P_r suggest that the chromophore in P_r adopts the C_{15}-*Z*,syn conformation.

Photomorphogenesis in plants is controlled by the biliprotein phytochrome as well as several blue and UV-absorbing photoreceptors (Furuya, 1987; Shropshire & Mohr, 1983; Pratt, 1982). The phytochrome photoreceptor from *Avena sativa* is a soluble cytoplasmic chromoprotein which consists of a dimer of 124-kDa subunits (Vierstra & Quail, 1986). Each subunit contains a single, thioether-linked, 2,3-dihydrobiliverdin prosthetic group (Lagarias & Rapoport, 1980; Rudiger, 1987; Vierstra & Quail, 1986). Phytochrome regulates photomorphogenesis in plants by adopting one of two photointerconvertible forms: P_r, which absorbs red light, and P_{fr}, which absorbs far-red light (Figure 1). Light absorption by P_r triggers the phototransformation to P_{fr}, a process that proceeds through at least two spectrally distinct intermediates. The production of P_{fr} is required for phytochrome-dependent photomorphogenesis.

The molecular changes in the chromophore and protein which occur in the P_r to P_{fr} phototransformation are not well understood. Biochemical probes of protein conformation using proteases, protein kinases, and monoclonal antibodies suggest that a significant conformational change accompanies the P_r to P_{fr} transformation (Cordonnier, 1989; Lagarias, 1985; McMichael & Lagarias, 1990). On the basis of NMR studies of chromopeptides, Rudiger et al. have proposed that a $Z \rightarrow E$ isomerization of the 2,3-dihydrobiliverdin prosthetic group occurs during the P_r to P_{fr} transition (Rudiger, 1987; Rudiger et al., 1983). This suggests that the primary photochemical reaction is a $Z \rightarrow E$ isomerization although alternatives have been discussed (Moon et al., 1985; Sarkar & Song, 1981).

Resonance Raman spectroscopy is a powerful method for the determination of chromophore structure in situ. However, because of the intrinsic fluorescence of phytochrome it has been difficult to obtain a Raman spectrum of this chromoprotein. We have recently shown that the resonance Raman spectrum of oat phytochrome in the P_r form can be obtained by using 752-nm laser excitation which is sufficiently red shifted to avoid the fluorescence emission of P_r (Fodor et al., 1988; Fodor et al., 1989). In conjunction with earlier Raman analyses on biliverdin dimethyl ester (Margulies & Toporowicz, 1984), our studies indicated that the linear tetrapyrrole chromophore of P_r is protonated in situ, thereby accounting at least in part for its red-shifted absorption. Qualitatively similar studies were subsequently reported by using coherent anti-Stokes Raman spectroscopy (CARS)[1] on rye phytochrome preparations (Hermann et al., 1990); however, surface-enhanced resonance Raman spectroscopy (SERRS) on oat phytochrome has afforded significantly different spectra (Farrens et al., 1989; Rospendowski et al., 1989). Here we extend our earlier observations by reporting the resonance Raman spectrum of the P_{fr} form of oat phytochrome. New bands are found for P_{fr} with frequencies and intensities that are distinctly different from those of P_r. These results, along with vibrational normal mode calculations and resonance Raman intensity calculations on a 2,3-dihydrobiliverdin model compound, support the idea that there is a C_{15} $Z \rightarrow E$ isomerization of the phytochrome chromophore in the P_r to P_{fr} phototransformation.

MATERIALS AND METHODS

Phytochrome was purified from 5-day-old etiolated oat (*A. sativa*) seedlings following Litts et al. (1983) according to the modifications of Lagarias and Mercurio (1985). The A_{668}/A_{280} ratios for the 124-kDa preparations were 0.6–1.0. For the P_{fr} experiments, the pigment was dissolved in a 50 mM EPPS,

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[1] Abbreviations: HOOP, hydrogen out of plane; EPPS, *N*-(2-hydroxyethyl)piperazine-*N*′-3-propanesulfonic acid; CARS, coherent anti-Stokes Raman scattering; SERRS, surface-enhanced resonance Raman scattering.

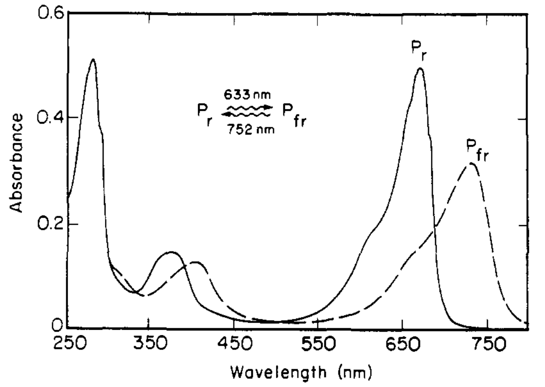


FIGURE 1: Absorption spectra of the P_r and P_{fr} forms of phytochrome adapted from Kelly and Lagarias (1985) and Lagarias et al. (1987). The P_r form of phytochrome absorbs light maximally a[illegible]3 nm while the P_{fr} form absorbs maximally at 730 nm. The [illegible] forms are photointerconvertible at room temperature.

pH 8.0 buffer containing 1 mM EDTA at a concentration of ~15 μM, giving a final optical density of ~2/cm at 668 nm. The 7-mL phytochrome sample was placed in a closed-loop recirculating flow system consisting of silicon tubing attached to a 1.5-mm inside diameter glass capillary. The sample was pumped with a Cole-Parmer Model 7535-20 peristaltic pump at a linear velocity of ~35 cm/s (temperature 20–22 °C).

To obtain room temperature resonance Raman spectra of P_{fr}, the flow capillary was illuminated with ~14 mW of cylindrically focused 752-nm light from a krypton ion laser. The reservoir was illuminated with ~30 mW of 633-nm light from a HeNe laser to keep the sample predominantly in the P_{fr} form. The dwell time between leaving the illuminated reservoir and arriving at the flow cell was ~700 ms. This delay is short enough to ensure that the predominantly P_{fr}-containing sample does not thermally relax to P_r before reaching the probe beam (Litts et al., 1983), and it is sufficiently long to permit any intermediates produced by the actinic illumination to decay to P_{fr} or P_r (Eilfeld et al., 1987). Since the 752-nm probe laser strongly overlaps with the P_{fr} absorption band (λ_{max} 730 nm), this method provides strong resonance enhancement of the scattering from P_{fr}. The back-conversion of P_{fr} to P_r by this probe laser depends upon the photoalteration parameter, $F = (3.824 \times 10^{-21})P\epsilon\phi/\nu d$ (Mathies et al., 1976), where P is the laser power (photons/s), ϵ is the molar absorption coefficient (M^{-1} cm^{-1}), ϕ is the quantum yield for the photochemical reaction, ν is the flow velocity (cm/s), and d is the focused beam diameter (cm). For P_{fr}, $\epsilon_{752} \cong 57\,000$ M^{-1} cm^{-1} and $\phi = 0.069$ (Lagarias et al., 1987), giving $F \sim 0.1$. This means that less than 10% of the P_{fr} molecules that flow through the probe laser beam will be photoconverted to P_r.

Room temperature resonance Raman spectra of P_r were obtained from an $(NH_4)_2SO_4$ precipitate of phytochrome suspended in a small amount of pH 8, 50 mM EPPS, and 1 mM EDTA buffer at a concentration of ~76 μM. This gives an optical density of ~10/cm at 668 nm. The sample was placed in a spinning 5-mm diameter glass optical cell as previously described (Fodor et al., 1988), except that it was maintained at room temperature. The sample was illuminated with ~20 mW of spherically focused (*f1* = 75 mm) 752-nm light.

All Raman data were acquired with a Spex 1401 double monochromator equipped with a Hammamatsu 943-02 photomultiplier and photon-counting detection system interfaced to a PDP 11/23 computer. The monochromator step size was 2 cm^{-1} with a 2-s dwell time, and the spectral band pass was ~6 cm^{-1}. Five scans were averaged for the P_{fr} spectrum and 12 scans for the P_r room temperature spectrum. The fluorescence background was subtracted by using a cubic spline fitting routine. The Raman data were corrected for the spectral sensitivity of the detection system.

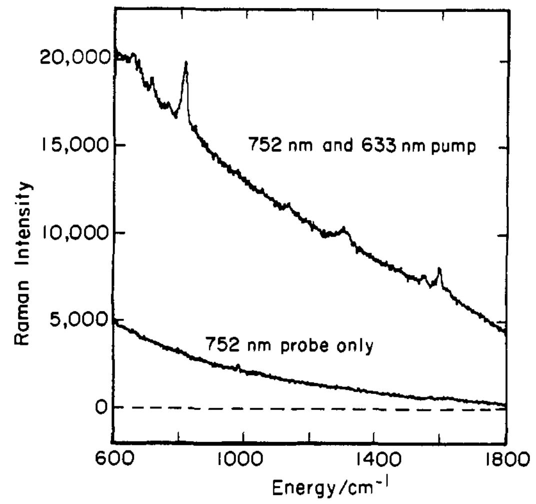
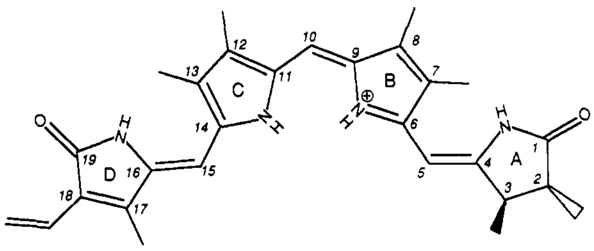


FIGURE 2: Method for obtaining P_{fr} spectra. The lower spectrum was recorded from a flowing phytochrome solution excited with 15 mW at 752 nm. In the top spectrum, the sample reservoir was simultaneously irradiated with a 633-nm pump beam to photoconvert the phytochrome from P_r to P_{fr}. The increase in the fluorescence background and the new vibrational features in the top spectrum are due to P_{fr}.

Chart I: C_{15}-*Z*,Anti, C_{10}-*Z*,Syn, C_5-*Z*,Anti 2,3-Dihydrobiliverdin (Structure I)



Vibrational calculations were performed on 2,3-dihydrobiliverdin with methyl groups at positions 3, 8, and 12 (structure I in Chart I). The full tetrapyrrole chromophore was necessary to correctly predict the dependence of the vibrations on structural changes. The initial geometry was adapted from the X-ray analysis of the tetrapyrrole conformation in C-phycocyanin (Schirmer et al., 1987). The initial structure used was *Z*,anti, *Z*,syn, *Z*,anti for the C_{15}, C_{10}, and C_5 methine bridges, respectively. The C_{15} *Z*,syn, *E*,syn, and *E*,anti conformers were generated by rotation about the $C_{15}{=}C_{16}$ or C_{14}–C_{15} bonds. As suggested by both the X-ray structure of C-phycocyanin (Schirmer et al., 1987) and the resonance Raman studies on P_r (Fodor et al., 1988), the chromophore used for these calculations was fully protonated on the pyrrole nitrogens. The geometries were then minimized and the vibrations calculated according to the QCFF/π method (Warshel & Karplus, 1974).

RESULTS

Our method for obtaining the resonance Raman spectrum of P_{fr} is illustrated in Figure 2. First, a flowing solution of phytochrome is illuminated with just the 752-nm probe light which is expected to keep phytochrome exclusively in the P_r form (Kelly & Lagarias, 1985; Lagarias et al., 1987). At the low concentrations used here, no resonance-enhanced vibra-

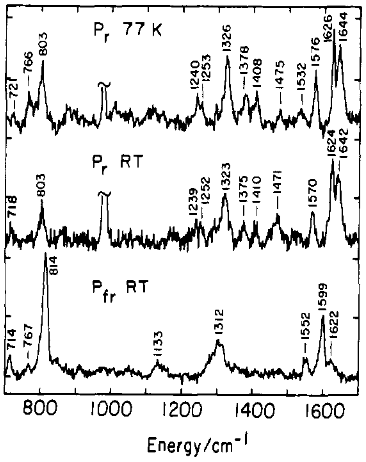


FIGURE 3: Comparison of the resonance Raman spectra of the P$_r$ and P$_{fr}$ forms of phytochrome (752-nm probe). The spectrum of P$_r$ at low temperature is from Fodor et al. (1988). The room temperature P$_r$ spectrum was obtained from a concentrated solution of phytochrome in a rotating cell. The signal-averaged P$_{fr}$ spectrum was obtained as described in Figure 2. The truncated band at ~983 cm^{-1} is due to sulfate.

tional bands from P$_r$ are observed. Only the tail of the broad fluorescence emission from P$_r$ is detected. Addition of the 633-nm pump beam to the sample reservoir converts P$_r$ to P$_{fr}$, causing an increase in the fluorescence background and the appearance of vibrational lines due to P$_{fr}$. The spectrum reverts to that shown in the bottom trace of Figure 2 when the 633-nm pump laser is blocked because P$_{fr}$ is converted back to P$_r$ by the 752-nm probe laser.

Figure 3 shows the signal-averaged P$_{fr}$ spectrum compared to the spectra for P$_r$ obtained at room temperature with the rotating cell (this work) and at low temperature (Fodor et al., 1988). The low-temperature and room temperature P$_r$ spectra are very similar. The prominent bands in the room temperature P$_r$ spectrum at 803, 1323, 1570, 1624, and 1642 cm^{-1} have nearly the same frequencies and intensities as those in the low-temperature P$_r$ spectrum. Comparison of the P$_r$ and P$_{fr}$ spectra show that their mode frequencies and intensities differ significantly. The bands in the C=C and C=N stretching regions in the room temperature P$_r$ spectrum at 1642, 1624, and 1570 cm^{-1} are replaced by bands at 1622, 1599, and 1552 cm^{-1} in the P$_{fr}$ spectrum. In addition, a strong band at 1323 cm^{-1} in P$_r$, which has been correlated with protonation of the chromophore (Fodor et al., 1988; Margulies & Toporowicz, 1979), shifts down to 1312 cm^{-1} in P$_{fr}$. The 803-cm^{-1} band in P$_r$ is replaced by a very intense band at 814 cm^{-1} in P$_{fr}$. The frequency of this mode suggests that it is either a skeletal bending mode or a hydrogen out-of-plane (HOOP) wag.

To provide initial information on the vibrational assignments of the P$_{fr}$ spectrum, we obtained its resonance Raman spectrum in D$_2$O buffer (Figure 4). In D$_2$O, the 1312-cm^{-1} band disappears and is replaced by a band at 1060 cm^{-1}. Therefore, the 1312-cm^{-1} band is assigned as an N–H rock which shifts to 1060 cm^{-1} upon deuteriation. The 1599-cm^{-1} C=C stretching mode shifts down to 1591 cm^{-1} upon deuteriation, consistent with the expected coupling of the N–H rocks with C=C stretches. The larger shift of the 1552-cm^{-1} band (presumably to 1494 cm^{-1}) in D$_2$O indicates that it is a C=N stretch. Finally, the small 3-cm^{-1} shift of the 814-cm^{-1} mode in D$_2$O shows that it is not an N–H HOOP and therefore must be a C–H HOOP that has weak coupling with N–H modes.

To help interpret these spectra, we performed vibrational calculations on the tetrapyrrole model compound I and its other three structural isomers at the C$_{15}$ methine bridge. The calculated vibrational frequencies and intensities for selected ethylenic stretching, N–H rocking, and HOOP wagging modes are presented in Figure 5. The indicated N–H modes are the only two normal modes which had a large amount of N–H rock character localized in them as evidenced by their large shift upon N-deuteriation. The QCFF/π method generally has difficulty predicting the intensity and frequency of nearly degenerate and highly delocalized ethylenic modes. While the ethylenic mode frequencies and intensities are somewhat sensitive to structure, no clearly interpretable pattern of frequency or intensity changes emerges. The N–H rocking modes are more sensitive to structure, the consistent trend being the lowering of the N–H frequencies in the C$_{15}$-*E* molecules. An analogous drop in the N–H rocking band is seen upon P$_r$ → P$_{fr}$ conversion (Figure 3). In addition, each of the isomers has a localized C$_{15}$ HOOP mode in the ~850-cm^{-1} region which

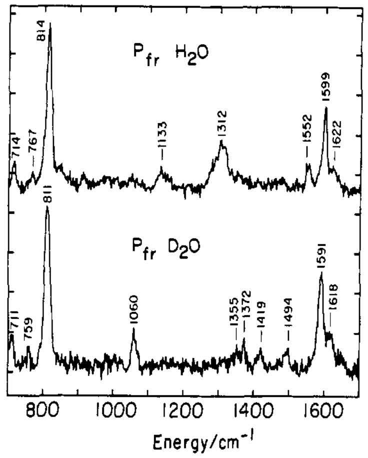


FIGURE 4: Comparison of the room temperature Raman spectra of P$_{fr}$ phytochrome in H$_2$O and D$_2$O. Spectra were obtained from a flowing solution of phytochrome at room temperature.

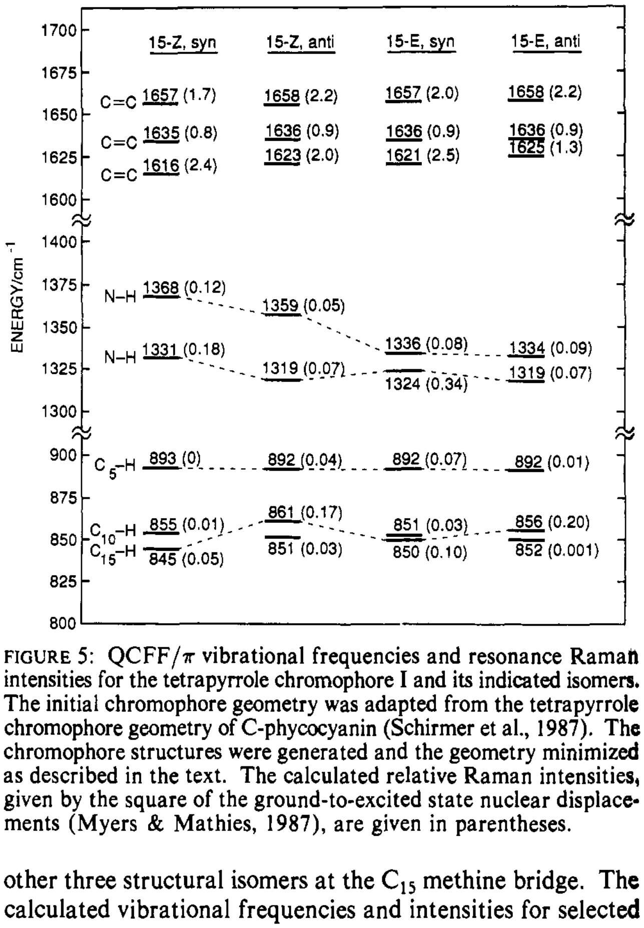
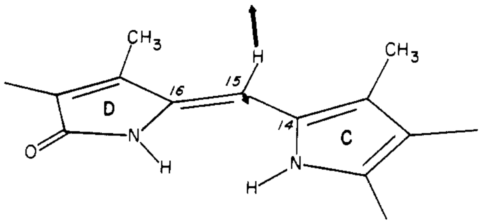
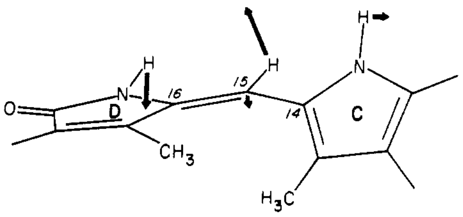


FIGURE 5: QCFF/π vibrational frequencies and resonance Raman intensities for the tetrapyrrole chromophore I and its indicated isomers. The initial chromophore geometry was adapted from the tetrapyrrole chromophore geometry of C-phycocyanin (Schirmer et al., 1987). The chromophore structures were generated and the geometry minimized as described in the text. The calculated relative Raman intensities, given by the square of the ground-to-excited state nuclear displacements (Myers & Mathies, 1987), are given in parentheses.



$C_{15}=C_{16}$ Z, $C_{14}-C_{15}$ syn

845 cm^{-1}



$C_{15}=C_{16}$ E, $C_{14}-C_{15}$ anti

856 cm^{-1}

FIGURE 6: Cartesian displacements for the C_{15} hydrogen out-of-plane vibration calculated by the QCFF/π method for the $C_{15}=C_{16}$ *Z*(cis), $C_{14}-C_{15}$ syn, and the $C_{15}=C_{16}$ *E*(trans), $C_{14}-C_{15}$ anti geometries.

has significant intensity and whose intensity and frequency are sensitive to structure. A frequency upshift and an increase in intensity are observed between the C_{15}-*Z*,syn structure and the other isomers. The C_{15} HOOP modes for the C_{15}-*Z*,syn and the C_{15}-*E*,anti structures are depicted in Figure 6. The other two methine bridge HOOP modes exhibit little resonance Raman intensity, and their frequencies are insensitive to the calculated structural changes around the C_{15}-methine bridge. Since P_r and P_{fr} both exhibit a strong mode in the HOOP region whose intensity and frequency is very sensitive to the P_r to P_{fr} interconversion, we tentatively assign this band as the C_{15} HOOP.

Torsional distortions around single or double bonds in π-conjugated systems are known to cause significant increases in the intensity of HOOP modes in resonance Raman spectra (Eyring et al., 1980). Due to steric interactions, the C_{15}-*E*,anti isomer must be more distorted from planarity than the other isomers. This distortion has a marked effect on the resonance Raman spectrum. As the torsional distortion around the $C_{14}-C_{15}$ bond is increased from ~20° in the $C_{15}=C_{16}$ *Z*, $C_{14}-C_{15}$ syn structure to ~48° in the $C_{15}=C_{16}$ *E*, $C_{14}-C_{15}$ anti structure, the calculated Raman intensity increases by a factor of ~4. In the $C_{15}=C_{16}$ *E*, $C_{14}-C_{15}$ anti isomer, substantial distortion is required about the $C_{14}-C_{15}$ and $C_{15}=C_{16}$ bonds because the methyl groups at positions 13 and 17 are in close proximity, preventing relaxation to a planar structure (see Figure 6). The C_{15} HOOP intensity is also significant for the C_{15}-*Z*,anti and C_{15}-*E*,syn structures where the methyl groups interact with the pyrrole N–H's. The unusually large intensity of the 814-cm^{-1} band in the P_{fr} spectrum, which we assign as the C_{15} HOOP, suggests that there is a high degree of torsional distortion of the tetrapyrrole chromophore.

DISCUSSION

The resonance Raman spectra of phytochrome presented here provide an in situ probe of the chromophore structural changes that occur upon P_r to P_{fr} interconversion. The frequencies and intensities of the vibrational bands are very different in P_r and P_{fr}. This shows that a major structural change has occurred in the chromophore geometry. In our earlier study of P_r (Fodor et al., 1988), we considered the possibility that the resonance Raman spectrum of P_r might contain some scattering from P_{fr} because the probe laser at 752 nm would preferentially excite P_{fr}. We dismissed this possibility due to the low levels of P_{fr} present in the sample under the experimental conditions employed. The absence of bands corresponding to P_{fr} in the resonance Raman spectra of P_r (see Figure 3) clearly confirms that there was no such contamination.

We interpret the spectral changes between P_r and P_{fr} to reflect isomerization at the C_{15} methine bridge from C_{15}-*Z*,syn (cis,syn) in P_r to C_{15}-*E*,anti (trans,anti) in P_{fr}. Our arguments in favor of this conclusion are as follows. First, calculations on the 2,3-dihydrobiliverdin models predict that a C_{15} $Z \rightarrow E$ isomerization should produce a general lowering of the N–H rocking modes. The bands at 1323 cm^{-1} in P_r and 1312 cm^{-1} in P_{fr} can be confidently assigned as N–H rocks on the basis of their deuteration shifts [this work and Fodor et al. (1988)]. Thus, the frequency drop of the N–H rocking band between P_r and P_{fr} suggests that a $Z \rightarrow E$ isomerization has taken place. Second, the intense vibrational mode which we have assigned as the C_{15} HOOP appears at a higher frequency and intensity in P_{fr} (814 cm^{-1}) than in P_r (803 cm^{-1}). Calculations on the model 2,3-dihydrobiliverdin chromophore with different C_{14}-C_{15} and $C_{15}=C_{16}$ structures suggest that an increase in both the frequency and intensity of the C_{15} HOOP mode in P_{fr} is consistent with the production of a structure with a more distorted conformation. The C_{15}-*Z*,anti and C_{15}-*E*,anti structures both have elevated C_{15} HOOP modes with strong intensity and are therefore possibilities for the geometry at the C_{15} methine bridge of P_{fr}. On the basis of the analysis of the N–H rocking modes presented here and NMR studies on P_{fr} chromopeptides (Rudiger et al., 1983), which both support the C_{15}-*E* configuration for the chromophore in P_{fr}, the C_{15}-*E*,anti geometry is the more consistent assignment. Furthermore, the particularly low frequency of the C_{15} HOOP mode in P_r along with its 11-cm^{-1} increase upon photoconversion to P_{fr} supports the assignment of the C_{15}-*Z*,syn geometry in P_r. *We therefore conclude that the P_r to P_{fr} phototransformation involves a C_{15}-Z,syn to C_{15}-E,anti structural change of the chromophore.*

Previous NMR studies on chromopeptides isolated from P_r and P_{fr} have supported the hypothesis that the phototransformation of phytochrome involves a C_{15}-*Z* to C_{15}-*E* isomerization (Rudiger, 1987; Rudiger et al., 1983). However, the non-native absorption spectra of these chromopeptides indicate that the chromophore is adopting modified conformations and/or protonation states (Rudiger, 1987). This is particularly true for the P_{fr} chromopeptide which has an absorption maximum shifted more than 100 nm to the blue of the native holoprotein. For these reasons, the NMR analyses cannot address the conformation and protonation state of the chromophore in the native chromoprotein. Our resonance Raman spectra provide in situ information about the configuration, conformation, and protonation state of the native prosthetic group in P_r and P_{fr} and indicate that there is a dual change in the $C_{15}=C_{16}$ double bond configuration and the $C_{14}-C_{15}$ single bond conformation in the P_r to P_{fr} phototransformation. However, the nature of the primary photochemical event cannot be determined from these data. The primary step could be a $C_{15}=C_{16}$ isomerization alone or a simultaneous C_{15}-*Z*,syn to C_{15}-*E*,anti isomerization.

On the basis of the ratio of the oscillator strength between the near-UV and red absorption bands for P_r, Song and coworkers have proposed a semicircular conformation for the

2,3-dihydrobiliverdin prosthetic group in situ (Song, 1988; Song et al., 1979). Other investigators have suggested that the long-wavelength absorption of P_r is due to a more extended conformation (C_5-*Z*,anti, C_{10}-*E*,anti, C_{15}-*Z*,anti) than that found in phycocyanin (Rudiger, 1987; van der Hoef et al., 1987; Falk, 1989). This conformation, which differs from that of phycocyanin at the C_{10} methine bridge, provides a potential explanation for the considerable red shift of the P_r spectrum compared with that of phycocyanin. This assignment would not change the interpretation of our resonance Raman data since the vibrational properties of the C- and D-ring dipyrrole unit are not very sensitive to the C_{10} methine bridge structure. Isomerization to a C_{15}-*E*,anti geometry in P_{fr}, as proposed here, will produce an even more extended chromophore structure which might be expected to have an enhanced red oscillator strength which is not observed. However, the optical properties of the chromophore in P_{fr} may be significantly different from those of planar model compounds because of the torsional distortion of the C_{15} methine bridge in the *E*,anti geometry (see Figure 6). It should be noted that the intense HOOP modes in P_{fr} are strikingly similar to those observed in bathorhodopsin, the primary photoproduct of vision, which are caused by conformational distortion of the retinal chromophore (Eyring et al., 1980; Palings et al., 1989).

Recently, Song and co-workers reported SERRS spectra of phytochrome on silver electrodes and on colloidal silver sols (Farrens et al., 1989; Rospendowski et al., 1989). On electrodes, they observed P_r and P_{fr} spectra that were indistinguishable from each other and which were significantly different from those reported here. This is not surprising since adsorption to a metal surface can significantly alter the native protein–chromophore interactions. Somewhat different vibrational spectra were observed for P_r and P_{fr} adsorbed to colloidal silver in blue probe experiments (Farrens et al., 1989; Rospendowski et al., 1989). However, these spectra bear little resemblance to the spectra of native P_r and P_{fr} reported here. On the other hand, the red probe SERRS spectrum of P_r on silver sols (Farrens et al., 1989) exhibits a number of the major bands observed in the resonance Raman spectrum of native P_r [this work and Fodor et al. (1989)]. This comparison allows one to conclude that the red probe SERRS data do contain scattering from P_r. Unfortunately, the analogous red-probe SERRS spectra of P_{fr} have not been reported, so it is not possible to make a critical comparison between the resonance Raman and SERRS data concerning the structural changes that occur in the P_r to P_{fr} transformation.

Now that conditions have been established for obtaining resonance Raman spectra of both forms of phytochrome, it should be possible to exploit recently developed procedures for the in vitro assembly of phytochrome (Elich et al., 1989; Lagarias & Lagarias, 1989) to study pigments regenerated with chromophores isotopically labeled at specific locations. It will be particularly important to study 15-deuterio chromophores to test the assignments made here. This should enable us to elucidate the relationship between the vibrational spectrum and chromophore structure using the same approach that has been successfully applied to retinal proteins (Mathies et al., 1987). Once conditions are defined for obtaining the resonance Raman spectra of the photointermediates in both the forward and reverse transitions between P_r and P_{fr}, it should also be possible to determine the structural changes associated with the phytochrome phototransformation process.

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CORRECTIONS

Structural Studies of Cytochrome b_5: Complete Sequence-Specific Resonance Assignments for the Trypsin-Solubilized Microsomal Ferrocytochrome b_5 Obtained from Pig and Calf, by R. D. Guiles, John Altman, James J. Lipka, Irwin D. Kuntz, and Lucy Waskell*, Volume 29, Number 5, February 6, 1990, pages 1276–1289.

James J. Lipka was omitted from the byline. He was affiliated with the Department of Anesthesia, University of California, and the Anesthesiology Service, Veterans Administration Medical Center, at the time of the research. His present address is Stanford University Blood Bank, Pathology Department, Stanford University, Stanford, CA 94305.

Structural Consequences of Effector Binding to the T State of Aspartate Carbamoyltransferase: Crystal Structures of the Unligated and ATP- and CTP-Complexed Enzymes at 2.6-Å Resolution, by Raymond C. Stevens, J. Eric Gouaux, and William N. Lipscomb*, Volume 29, Number 33, August 21, 1990, pages 7691–7701.

Pages 7697 and 7698. The legends for Figures 7 and 8 are reversed.

Control of N-Linked Oligosaccharide Synthesis: Cellular Levels of Dolichyl Phosphate Are Not the Only Regulatory Factor, by Y. T. Pan and Alan D. Elbein*, Volume 29, Number 35, September 4, 1990, pages 8077–8084.

Page 8083. In the last paragraph of column 2, sentence 4 should read as follows: In fact, studies by Kean (1982) with a microsomal enzyme preparation have shown that the addition of dolichyl-P-mannose stimulated incorporation of GlcNAc from UDP-[^{3}H]GlcNAc into GlcNAc-PP-dolichol.