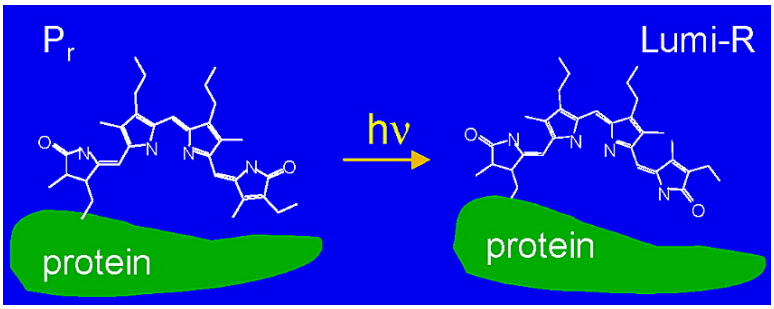
Communication

Determination of the Chromophore Structures in the Photoinduced Reaction Cycle of Phytochrome

Maria Andrea Mroginski, Daniel H. Murgida, David von Stetten, Christa Kneip, Franz Mark, and Peter Hildebrandt

J. Am. Chem. Soc., **2004**, 126 (51), 16734-16735• DOI: 10.1021/ja043959l • Publication Date (Web): 02 December 2004



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 7 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 12/02/2004

Determination of the Chromophore Structures in the Photoinduced Reaction Cycle of Phytochrome

Maria Andrea Mroginski,*,† Daniel H. Murgida,† David von Stetten,† Christa Kneip,‡ Franz Mark,‡ and Peter Hildebrandt*,†

Technische Universität Berlin, Institut für Chemie, Sekr. PC 14, Strasse des 17. Juni 135, D-10623 Berlin, Germany, and Max-Planck-Institut für Bioanorganische Chemie, Stiftstrasse 34-36, D-45470 Mülheim, Germany

Received October 4, 2004; E-mail: hildebrandt@chem.tu-berlin.de

Phytochromes constitute a family of sensory photoreceptors that are ubiquitous in plants and have been recently also discovered in bacteria.[1] The chromophoric site is constituted by a linear methine-bridged tetrapyrrole (Figure 1) that is covalently attached to the apoprotein. Upon light absorption, phytochrome runs through a reaction sequence from the inactive form P_r to the active form P_{fr} triggering the signal transduction pathway. It is commonly accepted that the photoprocess involves a $Z \rightarrow E$ isomerization of the methine bridge between the rings C and D.[2] However, the three-dimensional structure of phytochrome is not yet available, and even for the stable states sound information about details of the chromophore structure has not been obtained thus far. In this respect, resonance Raman (RR) spectroscopy is an indispensable technique since it exclusively probes the vibrational bands of the tetrapyrrole. However, there is no consensus on the interpretation of the RR spectra that have been obtained for the parent states and the photocycle intermediates.[3,4]

In this contribution we present a combined experimental and theoretical approach to determine the phytochromobilin (PΦB) structure from the RR spectra of phytochrome phyA (oat) by comparison with Raman spectra calculated for different tetrapyrrole geometries. Vibrational spectra were obtained by density functional theory (DFT) using the B3LYP functional[5] and the 6-31G* basis set. The force field was scaled by a set of global scaling factors determined for a series of model compounds including hydrogen-bonded systems.[6] This approach affords an accuracy of ± 11 cm^{-1} for the calculated frequencies. Calculated Raman intensities that serve as additional assignment criteria agree qualitatively with the experimental data[6] and, moreover, provide a good description for the experimental spectra of tetrapyrroles measured with preresonant excitation.[4b,7]

For open-chain tetrapyrroles, the number of isomers that differ with respect to the methine bridge configuration (*Z*/*E*) and conformation [*syn*(*s*)/*anti*(*a*)] is 64 (Figure 1). In 32 geometries, the C−D methine bridge is in the *Z* configuration as it is most likely to be in the P_r state.[2] Furthermore, the P_r chromophore probably exhibits an extended structure.[8] Thus, we have sorted out isomers with largely helical structures and isomers exhibiting highly distorted geometries due to steric interactions. The remaining 15 isomers that include also those previously proposed for the chromophore structure in P_r,[3,4,7] constitute the set of geometries for the DFT calculations. In each of these methine bridge isomers, the side chains of the pyrrole rings can adopt different conformations. As analyzed for a few cases, different propionic side-chain conformations are reflected in the Raman spectrum only below 1000 cm^{-1}, whereas variations of the methine bridge isomerization affect the spectrum also and quite markedly between 1000 and 1700 cm^{-1} (vide infra). Also esterification of the propionic acid side chains

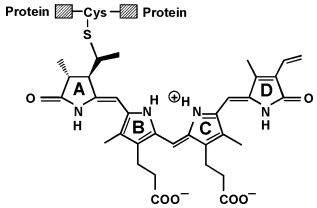


Figure 1. Phytochromobilin (PΦB) in the *ZZZasa* geometry.

caused only small spectral changes exclusively in the low frequency region. For all further calculations the methyl ester forms were used to avoid intramolecular hydrogen-bond interactions in the tetrapyrrole, which most likely do not exist in phytochrome. Significant interactions with the protein are expected for the N−H groups that are likely to form hydrogen bonds with adjacent amino acid residues. Since RR spectroscopy has revealed that all nitrogens are protonated in each state of phytochrome detected thus far,[4] a counterion has been included in the calculations. In phytochrome, the counterion has not yet been identified, but it is likely to be a carboxylate group. However, calculations with acetate and Cl^- ion yield very similar Raman spectra such that we have used a Cl^- ion as the simpler variant in all calculations. Finally, the thioether function (ring A), which constitutes the covalent linkage to the protein, is replaced by an ethyl group in the calculations. Using these approximations, we have optimized the geometries of more than 20 methine bridge isomers. For calculating the Raman spectra, standard scaling factors were employed[6a] except for the N−H internal coordinates for which the scaling factors of the hydrogen-bonded hexamethyl pyrromethene monomer were adopted.[6b]

Experimental RR spectra of the various states of phytochrome phyA (oat), which exhibit absorption maxima between 660 and 720 nm, were obtained with 1064-nm excitation at low temperature as described previously.[4] Under these preresonance conditions, Raman signals of the apoprotein only contribute to broad and weak humps in the spectrum.

In the region above 1000 cm^{-1}, the Raman spectrum of the *ZZZasa* isomer agrees very well with the experimental RR spectrum of P_r as shown for the range between 1490 and 1700 cm^{-1} in Figure 2. Thus, all of the observed RR bands can readily be assigned to the calculated bands. Relative intensities are well reproduced, and the frequency deviations are within the error found for model compounds.[6] This is also true for the chromophore deuterated at the pyrrole nitrogens (Figure 2). For all other methine bridge isomers, the agreement with the experimental spectra is much worse as illustrated for the *ZEZaas* geometry which has been previously proposed to be the chromophore conformation in P_r (see Supporting Information).[3b] Hence, we conclude that in P_r the chromophore is in the *ZZZasa* configuration, thereby confirming previous suggestions.[4,7] The results further reveal that the frequency range between

† Technische Universität Berlin.
‡ Max-Planck-Institut für Bioanorganische Chemie.

10.1021/ja043959l CCC: $27.50

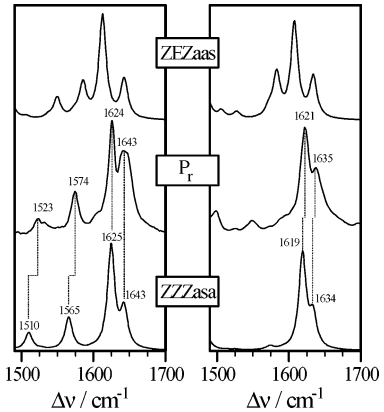


Figure 2. Calculated Raman spectra for the *ZEZsaa* and *ZZZasa* geometries of PΦB nondeuterated (left) and deuterated at the pyrrole nitrogens (right) and the experimental RR spectra of P_r in H_2O and D_2O.

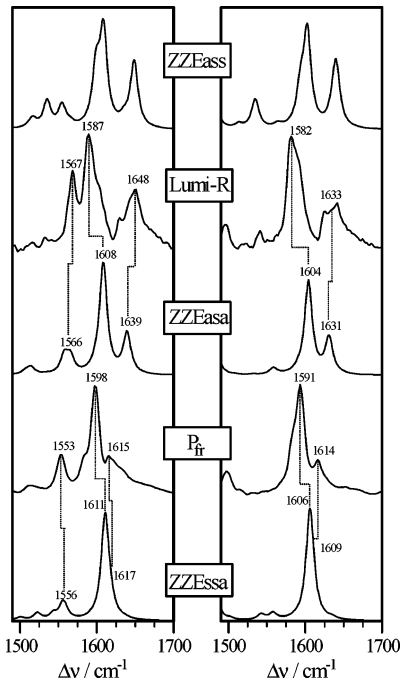


Figure 3. Calculated Raman spectra for the *ZZEaas*, *ZZEasa*, and *ZZEssa* geometries of PΦB nondeuterated (left) and deuterated at the pyrrole nitrogens (right) and the experimental RR spectra of Lumi-R and P_{fr} in H_2O and D_2O.

1000 and 1700 cm^{-1} is a characteristic fingerprint solely for the methine bridge isomerization of the tetrapyrrole. The region below 1000 cm^{-1} is likely to reflect more subtle conformational differences such as side-chain orientations (vide supra) and slight methine bridge torsions, which as well as electrostatic effects may be brought about by the interactions with the protein environment. Thus, it is not surprising that the deviations from the experimental spectra are somewhat larger in this region specifically for the C−H out-of-plane modes of the methine bridges.

The first step of the photocycle is the $Z \rightarrow E$ double bond isomerization of the C−D methine bridge,[2] suggested to be associated with a simultaneous rotation of the adjacent single bond.[3a] The Raman spectra calculated for the *ZZEasa* and *ZZEass* geometries both describe the experimental RR spectra of Lumi-R in a satisfactory manner (Figure 3). However, the transition to the final state P_{fr} is associated with substantial changes in the experimental RR spectra, which thus bear no resemblance to the calculated Raman spectra of these two geometries. The most pronounced spectral change is the 33 cm^{-1} (19 cm^{-1} in D_2O) downshift of the Lumi-R band at 1648 cm^{-1} (1633 cm^{-1} in D_2O). This band originates from the C═C stretching of the A−B methine bridge, indicating that this part of the tetrapyrrole undergoes a conformational change upon formation of P_{fr}. In fact, only upon single bond rotation at the A−B methine bridge (*ZZEssa*) a substantial downshift is calculated for this mode, which then comes in close proximity to the prominent methine stretching of the C−D bridge. For all other *ZZE* isomers the calculated frequency difference between both modes is (more than) 2 times larger than in the experimental spectra. Furthermore, in the entire spectral range, the calculated spectrum of the *ZZEssa* isomer provides an acceptable description of the experimental RR spectra of P_{fr} in the nondeuterated and deuterated form (see Supporting Information). Since for the Lumi-R $\rightarrow$ P_{fr} transition two thermal methine bridge single bond rotations are highly unlikely, the *ZZEssa* geometry in P_{fr} is only consistent with a *ZZEasa* configuration of the Lumi-R chromophore.

Thus, the reaction cycle of phytochrome is initiated by the *ZZZasa* (P_r) $\rightarrow$ *ZZEasa* (Lumi-R) photoisomerization followed by thermal relaxation steps including at least a partial $a \rightarrow s$ single bond rotation at the methine bridge A−B. This latter step, which may account for the change in hydrogen bonding of the C═O group of ring A,[9] most likely occurs with the formation of the precursor of P_{fr}, Meta-R_c, in view of the far reaching spectral similarities between Meta-R_c and P_{fr}.[4b] Elucidation of further structural details of the chromophore beyond the level of methine bridge isomerization requires more advanced theoretical methods that can take into account also the protein environment. Nevertheless, the present study is also an important step toward the development of a systematic strategy for determining molecular structures on the basis of vibrational spectra.

Acknowledgment. The work was supported by the Deutsche Forschungsgemeinschaft (Sfb 498, B9).

Supporting Information Available: Calculated Raman spectra for 21 methine bridge isomers of PΦB. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Quail, P. H. *Nat. Rev. Mol. Cell Biol.* **2002**, *3*, 85. (b) Hughes, J.; Lamparter, T. *Plant Physiol.* **1999**, *121*, 1059. (c) Fankhauser, C. *J. Biol. Chem.* **2001**, *276*, 11453.
(2) Rüdiger, W.; Thümmler, F.; Cmiel, E.; Schneider, S. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 6244.
(3) (a) Andel, F., III; Lagarias, J. C.; Mathies, R. A. *Biochemistry* **1996**, *35*, 15997. (b) Andel, F., III; Murphy, J. T.; Haas, J. A.; McDowell, M. T.; van der Hoef, I.; Lugtenburg, J.; Lagarias, J. C.; Mathies, R. A. *Biochemistry* **2000**, *39*, 2667. (c) Tokutomi, S.; Sugimoto T.; Mimuro, M. *Photochem. Photobiol.* **1992**, *56*, 545. (d) Mizutani, Y.; Tokutomi, S.; Kitagawa, T. *Biochemistry* **1994**, *33*, 153.
(4) (a) Matysik, J.; Hildebrandt, P.; Schlamann, W.; Braslavsky; S. E.; Schaffner, K. *Biochemistry* **1995**, *34*, 10497. (b) Kneip, C.; Hildebrandt, P.; Schlamann, W.; Braslavsky, S. E.; Mark, F.; Schaffner, K. *Biochemistry* **1999**, *38*, 15185.
(5) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5684.
(6) (a) Magdó, I.; Németh, K.; Mark, F.; Hildebrandt, P.; Schaffner, K. *J. Phys. Chem. A* **1999**, *103*, 289. (b) Mroginski, M. A.; Németh, K.; Magdó, I.; Müller, M.; Robben, U.; Della Védova, C.; Hildebrandt, P.; Mark, F. *J. Phys. Chem. B* **2000**, *104*, 10885.
(7) Kneip, C.; Németh, K.; Mark, F.; Hildebrandt, P.; Schaffner, K. *Chem. Phys. Lett.* **1999**, *311*, 479.
(8) Eilfeld, P.; Rüdiger, W. *Z. Naturforsch., C: Biosci.* **1985**, *40*, 109.
(9) Foerstendorf, H.; Benda, C., Gärtner, W.; Storf, M.; Scheer, H.; Siebert, F. *Biochemistry* **2001**, *40*, 14952.

JA043959L