BIOCHEMISTRY
including biophysical chemistry & molecular biology

Rapid Report

pubs.acs.org/biochemistry

On the Collective Nature of Phytochrome Photoactivation

Chen Song,[†] Georgios Psakis,[‡] Christina Lang,[‡] Jo Mailliet,[‡] Jan Zaanen,[§] Wolfgang Gärtner,[∥] Jon Hughes,[‡] and Jörg Matysik*,[†]

[†]Leids Instituut voor Chemisch Onderzoek, Universiteit Leiden, P.O. Box 9502, 2300 RA Leiden, The Netherlands

[‡]Pflanzenphysiologie, Justus-Liebig-Universität, Senckenbergstraße 3, D-35390 Giessen, Germany

[§]Instituut-Lorentz for Theoretical Physics, Universiteit Leiden, P.O. Box 9506, 2300 RA Leiden, The Netherlands

[∥]Max-Planck-Institut für Bioanorganische Chemie, Stiftstraße 34-36, D-45470 Mülheim an der Ruhr, Germany

Supporting Information

ABSTRACT: The red/far-red-sensing biological photoreceptor phytochrome is a paradigmatic two-state signaling system. The two thermally stable states are interconverted via a photoreaction of the covalently bound tetrapyrrole chromophore. Applying recently developed solid-state nuclear magnetic resonance, we study both the chromophore and its protein pocket in the Pr (red-absorbing) and Pfr (far-red-absorbing) states. The observations show that the phototransformation combines local chemical reactions with a mesoscopic transition of order. Both the chromophore and its binding pocket are quasi-liquid and disordered in Pr, yet quasi-solid and ordered in Pfr. Possible biochemical implications are discussed.

Phytochrome photoreceptors were first identified in plants as major regulatory elements in photomorphogenesis.[1] Subsequently, more primitive phytochromes were identified and characterized in prokaryotes.[2−4] Phytochromes accomplish their function with the help of a covalently bound open-chain tetrapyrrole chromophore that interacts with the surrounding protein[1] (Figure 1). In general, assembly of the apoprotein with the chromophore in the dark forms the resting state (Pr; λ_{max} = 658 nm in the case of Cph1). Cph1 phytochrome, a light-regulated histidine kinase in cyanobacteria, comprises a sensory module, Cph1Δ2, which consists of the N-terminal Per/Arnt/Sim (PAS) domain, the chromophore-binding cGMP phosphodiesterase/adenylyl cyclase/FhlA (GAF) domain, and the phytochrome-associated (PHY) domain. The change of the signaling state is induced by red light absorption photoconverting phytochromes to the Pfr state (λ_{max} = 702 nm for Cph1). The underlying chemical process following photon absorption is an isomerization of the chromophore[5] occurring at the C15═C16 double bond.[6,7] The available crystal[8−11] and nuclear magnetic resonance (NMR)[7] structures of Pr and Pfr states provide only clues about the intramolecular functional mechanism used by the photoreceptor. Signaling is thought to involve residue Asp207, hydrogen-bonded to pyrrole ring ***D*** of the chromophore, and Arg254, which interacts with the ***B***-ring propionate side chain[7] (cyan and blue dotted arrows, respectively, in Figure 1C).

Here we investigate the mechanism of light-induced signaling of the chromophore within the Cph1Δ2 sensory module (residues 1−514) in greater detail by means of high ^{1}H-resolved magic-angle spinning (MAS) NMR spectroscopy. Recently, improved ^{1}H pulse schemes such as the windowed phase-modulated Lee−Goldburg (*w*PMLG3^{+}) homonuclear decoupling scheme[12] have been introduced, allowing for superb ^{1}H spectral resolution in the solid state. We therefore introduce[7] this scheme to the two-dimensional (2D) ^{1}H−^{13}C medium- and long-distance heteronuclear correlation (MELODI−HETCOR) technique.[13] This method is especially suitable

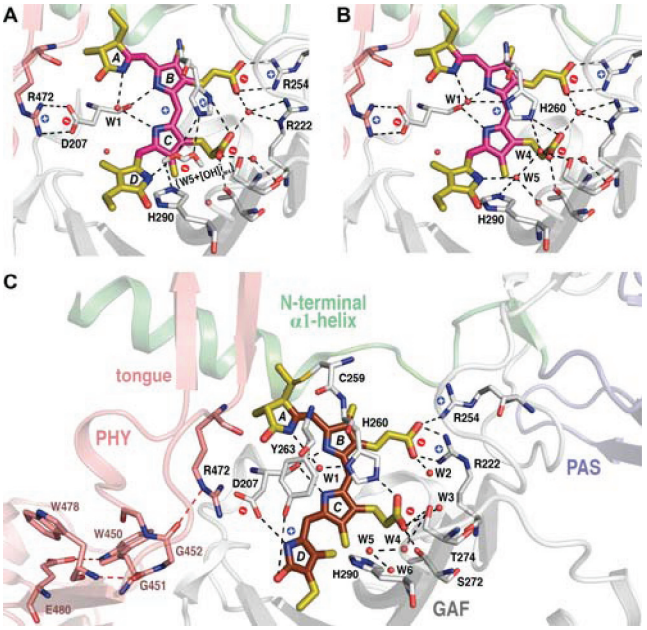

Figure 1. Structural views of the PCB-binding site showing the chromophore and representative ^{1}H contacts observed in MELODI−HETCOR spectra as two Pr isoforms [(A) **Pr-I** and (B) **Pr-II**] and Pfr (C). The presentations for Pr (A and B) and Pfr (C) are modeled according to the Cph1 2VEA and *Pa*BphP 3C2W structures, respectively, except that the chromophore adopts a *β*-facial ***D***-ring disposition in Cph1 Pfr. The chromophore is colored yellow. Black dashed lines highlight the hydrogen-bonding networks. The plus and minus signs represent the positive and negative charges, respectively. For clarity, *α*8-helix of the GAF domain has been omitted.

Received: September 27, 2011
Revised: November 29, 2011
Published: November 29, 2011

for exploring local proton environments and therefore charge distributions in large proteins.[7] For NMR measurements, Cph1Δ2 was assembled in vitro with a uniformly ^{13}C- and ^{15}N-labeled phycocyanobilin (PCB) chromophore. Both the Pr ground state and Pfr photoactivated state were investigated at 100% occupancy.[7]

Our main result is depicted in Figure 2A. The 2D $^{1}H-^{13}C$ interfacial correlation spectrum displays the ^{13}C chemical shift

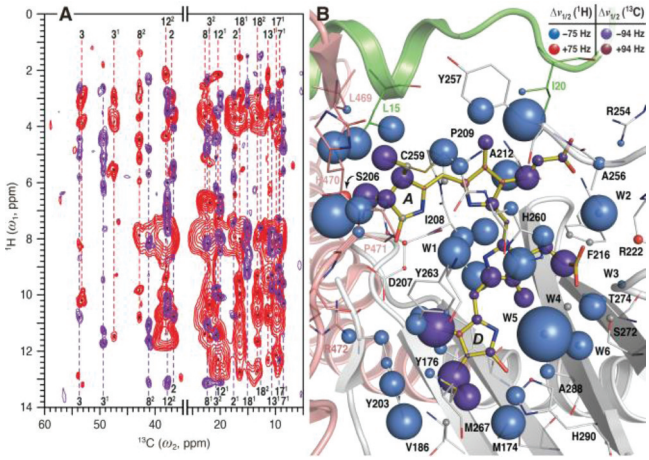

Figure 2. (A) Contour plot of the 2D MELODI–HETCOR spectra of *u*-[^{13}C,^{15}N]-PCB-Cph1Δ2 as Pr (red) and Pfr (lilac) revealing the intermolecular proximity between protons bound to amino acids and chromophore carbons. Chemical shifts of PCB carbons are indicated by dashed vertical lines (red for Pr and lilac for Pfr). For ^{13}C spectra, only the characteristic spectral region of 5–60 ppm is shown (for full spectra with assignments, see ref 7). (B) Structural representation of the FWHM (full-width at half-maximum) changes ($\Delta\nu_{1/2}$) in ^{13}C resonances of the chromophore and in its interfacial ^{1}H contacts. The size of the spheres refers to a Pfr minus Pr (**Pr-II**) $\Delta\nu_{1/2}$ of 75 Hz (as colored in red and blue for amino acids) and of 94 Hz (in raspberry and purple for the chromophore) as a PyMOL vdw parameter of 0.83. Gray spheres represent $\Delta\nu_{1/2}$ values of less than ±5%.

axis of the chromophore carbons in the ω_2-dimension (for assignment, see refs 6 and 7) and the ^{1}H chemical shifts of the protons bound to the amino acids around the chromophore in the ω_1-dimension (within approximately 5.5 Å). The most obvious difference between the Pr state (red) and the Pfr state (lilac) is the line width of the signals in both dimensions (expressed as half-height full width, $\nu_{1/2}$, in Figure 2B). All Pfr signals are sharp, indicating a well-defined and solid structure of the chromophore and its binding pocket. On the other hand, the Pr signals show a large variation in line widths (Tables S1 and S2 of the Supporting Information). Hence, the interaction of the chromophore with the surrounding amino acids in the Pr state is much weaker than in Pfr. In the Pr state, the chromophore is apparently loosely embedded in a soft pocket, kept in place by electrostatic and hydrogen-bonding interactions as well as by its covalent bond to Cys259.[14]

The data analysis also reveals that the chromophore exhibits more proton contacts in the "hard" Pfr state than in "soft" Pr state. The loss of signal intensity in soft matter is caused by motional averaging of the dipolar interactions and emphasizes the change in rigidity during the transition. Hence, the Pr-to-Pfr phototransformation is linked to a change in rigidity around the chromophore (Figure 2B and Figure S1 of the Supporting Information). The data demonstrate that the light-induced change in order and mobility affects a substantial volume. In fact, the resonances of 30 of 32 detected ^{13}C atoms and 51 of 58 ^{1}H atoms are broadened (Tables S1 and S2 of the Supporting Information). Hence, the photon absorption causes collective changes throughout the entire detection region.

The determination of the protonation and hydrogen-bonding state for each residue allowed the charge distribution for both Pr (Figure 1A,B) and Pfr (Figure 1C) to be reconstructed. The analysis also shows the coexistence of two isoforms in the Pr state distinguished by their charge distribution (**Pr-I** in Figure 1A and **Pr-II** in Figure 1B). The occurrence of two isoforms in one defined protein state demonstrates that the transition is associated with a local increase in the degree of order and is consistent with the idea of a liquid-to-solid transition. Also, the large area affected by the bifurcation of the Pr state (**Pr-I** and **Pr-II**) suggests an interpretation in terms of a collective transition.

These experimental observations are in accord with various earlier findings for phytochromes. Raman data implied that various bonds in the chromophore are strained in Pfr but not in Pr.[15] The Pr UV–vis absorbance spectra are not perturbed significantly by changing amino acids surrounding the chromophore, while significant effects are seen in the less permissive Pfr state.[16] Interestingly, however, such an effect was observed neither in the more primitive bacteriophytochrome Agp1[14] nor in a GAF-domain fragment of the "*SyB*-Cph1" phytochrome from *Synechococcus* OSB′,[17] implying that the observed collective changes are a derived feature.

Several possibilities exist with regard to the biological role of the change in dynamics observed on the mesoscopic scale. One is that the unusual flexibility of Pr might help insulate the protein and its downstream signaling system from noise deriving from thermal movements of the chromophore in darkness.[18] Another possibility is that the mesoscopic process itself is a part of the intramolecular signaling mechanism. A modification of microfluidity would affect the microscopic domain mechanics that has been proposed to control the signaling machinery.[19]

In protein biophysics, changes of disordered to ordered states are well-known. Examples of cofactor-induced order are given by metal or receptor binding in otherwise empty and therefore disordered pockets.[20] Disorder-to-order transitions in the N- and C-termini of hemoglobin have been associated with oxygen binding and release.[21] Entire proteins are known to undergo functional order-to-disorder changes upon interaction.[22] In the literature, such transitions are understood mostly in a chemical picture, as a result of particular chemical bond formations changing the mesoscopic domain mechanics but not the microscopic state of the matter. On the other hand, regular physical phase transitions in proteins have been reported, for example, at approximately 180 K affecting the long-range electron transfer in photosynthetic reaction centers.[23] Lacking a macroscopic periodicity, such phase transitions resemble liquid-to-glass transitions dramatically changing the time scale of long-range changes.

The phytochrome phototransformation indeed shows some characteristics of chemical reactions, in particular the double-bond isomerization as the local phototrigger and the occurrence of intermediate states that can be isolated at low temperatures.[15,24] On the other hand, the collectivity reminds us of the solidification of water to ice where, compared to chemical changes, relatively tiny dynamical changes in the whole aggregate occur collectively driven by nonlocal forces.

The light induction is reminiscent of organic molecular crystals undergoing phototriggered neutral-to-ionic phase transitions.[25] It appears that the transformation observed here comprises features of both chemical reactions and such physicochemical order transitions. The transition occurs on the mesoscopic scale and involves therefore aspects of both short-range chemical bond formation and long-distance solid-state transformation. A possible interpretation would be that the role of a control parameter (in phase transitions often the temperature) is taken up by a local chemical reaction.

The following questions remain: whether the origin of the observed mesoscopic transformation is sufficiently described by collective mechanical dynamics (as in a liquid-to-glass transition) or whether also electrodynamics is involved. A gas-to-liquid van-der-Waals transition, for example, requires a certain density of electric dipoles to induce collective behavior. We have shown that each of the three forms in Figure 1 has a quite distinct charge distribution and hydrogen-bonding network. The observed mesoscopic charge redistribution is likely to be characteristic of the mesoscopic transformation, relying on the changes in long-range forces such as Coulomb interactions. That would imply that the transition is associated with a local change in capacitance. For such an event, the matter should be at the verge of a polar-to-neutral transition[26] where cooperative proliferation of electric dipoles is the culprit, rather than translational freezing. We wonder whether the phenomenon is more ubiquitous in protein chemistry. Advanced MAS NMR spectroscopy affording highly resolved ^{1}H spectral resolution provides an ideal tool for settling this question.

■ ASSOCIATED CONTENT

Supporting Information

Sample preparation, experimental details, and supplementary figure and tables. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: (+31) 71 527 4198. E-mail: j.matysik@chem.leidenuniv.nl.

Funding

This work was supported by the *Volkswagen−Stiftung* (I/82628) (to W.G., J.H., and J. Matysik).

■ ACKNOWLEDGMENTS

This paper is dedicated to Prof. Kurt Schaffner on the occasion of his 80th birthday. We thank Profs. S. Vega (Weizmann Institute of Science, van Arkel guest Professor 2010 at Universiteit Leiden), H. Schiessel (Universiteit Leiden), and J. Neugebauer (Technische Universität Braunschweig) for fruitful discussions. Support from F. Lefeber, K. B. Sai Sankar Gupta, and K. Erkelens during various stages of the experiments is gratefully acknowledged.

■ REFERENCES

(1) Rockwell, N. C., Su, Y.-S., and Lagarias, J. C. (2006) *Annu. Rev. Plant Biol. 57*, 837−858.

(2) Hughes, J., Lamparter, T., Mittmann, F., Hartmann, E., Gärtner, W., Wilde, A., and Börner, T. (1997) *Nature 386*, 663.

(3) Yeh, K.-C., Wu, S.-H., Murphy, J. T., and Lagarias, J. C. (1997) *Science 277*, 1505−1508.

(4) Davis, S. J., Vener, A. V., and Vierstra, R. D. (1999) *Science 286*, 2517−2520.

(5) Braslavsky, S. E., Gärtner, W., and Schaffner, K. (1997) *Plant Cell Environ. 20*, 700−706.

(6) Rohmer, T., Lang, C., Hughes, J., Essen, L.-O., Gärtner, W., and Matysik, J. (2008) *Proc. Natl. Acad. Sci. U.S.A. 105*, 15229−15234.

(7) Song, C., Psakis, G., Lang, C., Mailliet, J., Gärtner, W., Hughes, J., and Matysik, J. (2011) *Proc. Natl. Acad. Sci. U.S.A. 108*, 3842−3847.

(8) Wagner, J. R., Zhang, J., Brunzelle, J. S., Vierstra, R. D., and Forest, K. T. (2007) *J. Biol. Chem. 282*, 12298−12309.

(9) Yang, X., Stojković, E. A., Kuk, J., and Moffat, K. (2007) *Proc. Natl. Acad. Sci. U.S.A. 104*, 12571−12576.

(10) Essen, L.-O., Mailliet, J., and Hughes, J. (2008) *Proc. Natl. Acad. Sci. U.S.A. 105*, 14709−14714.

(11) Yang, X., Kuk, J., and Moffat, K. (2008) *Proc. Natl. Acad. Sci. U.S.A. 105*, 14715−14720.

(12) Leskes, M., Madhu, P. K., and Vega, S. (2006) *J. Chem. Phys. 125*, 124506.

(13) Yao, L., Schmidt-Rohr, K., and Hong, M. (2001) *J. Magn. Reson. 149*, 139−143.

(14) Röben, M., Hahn, J., Klein, E., Lamparter, T., Psakis, G., Hughes, J., and Schmieder, P. (2010) *ChemPhysChem 11*, 1248−1257.

(15) Matysik, J., Hildebrandt, P., Schlamann, W., Braslavsky, S. E., and Schaffner, K. (1995) *Biochemistry 34*, 10497−10507.

(16) Hahn, J., Strauss, H. M., Landgraf, F. T., Gimenèz, H. F., Lochnit, G., Schmieder, P., and Hughes, J. (2006) *FEBS J. 273*, 1415−1429.

(17) Ulijasz, A. T., Cornilescu, G., Cornilescu, C. C., Zhang, J., Rivera, M., Markley, J. L., and Vierstra, R. D. (2010) *Nature 463*, 250−254.

(18) Gensch, T., Churio, S., Braslavsky, S. E., and Schaffner, K. (1996) *Photochem. Photobiol. 63*, 719−725.

(19) Möglich, A., and Moffat, K. (2010) *Photochem. Photobiol. Sci. 9*, 1286−1300.

(20) Chatterjee, C., Kumar, S., Chakraborty, S., Tan, Y. W., Leung, K. Y., Sivaraman, J., and Mok, Y.-K. (2010) *PLoS One 6*, e19208.

(21) Vergara, A., Vitagliano, L., Merlino, A., Sica, F., Marino, K., Verde, C., di Prisco, G., and Mazzarella, L. (2010) *J. Biol. Chem. 285*, 32568−32575.

(22) Dunker, A. K., and Kriwacki, R. W. (2011) *Sci. Am. 304*, 68−73.

(23) Kriegl, J. M., and Nienhaus, G. (2004) *Proc. Natl. Acad. Sci. U.S.A. 101*, 123−128.

(24) Rohmer, T., Lang, C., Bongards, C., Gupta, K. B. S. S., Neugebauer, J., Hughes, J., Gärtner, W., and Matysik, J. (2010) *J. Am. Chem. Soc. 132*, 4431−4437.

(25) Collet, E., Lemée-Cailleau, M. H., Buron-Le Cointe, M., Cailleau, H., Ravy, S., Luty, T., Bérar, J. F., Czarnecki, P., and Karl, N. (2002) *Europhys. Lett. 57*, 67−73.

(26) Torrance, J. B., Vazquez, J. E., Mayerle, J. J., and Lee, V. Y. (1981) *Phys. Rev. Lett. 46*, 253−257.