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Ubiquitous Structural Signaling in Bacterial Phytochromes

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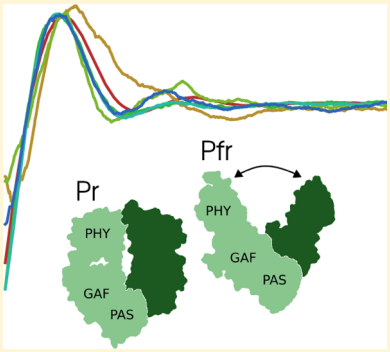
Supporting Information

ABSTRACT: The phytochrome family of light-switchable proteins has long been studied by biochemical, spectroscopic and crystallographic means, while a direct probe for global conformational signal propagation has been lacking. Using solution X-ray scattering, we find that the photosensory cores of several bacterial phytochromes undergo similar large-scale structural changes upon red-light excitation. The data establish that phytochromes with ordinary and inverted photocycles share a structural signaling mechanism and that a particular conserved histidine, previously proposed to be involved in signal propagation, in fact tunes photoresponse.



Phytochromes are light-sensing proteins found in plants, fungi, and bacteria. These dimeric photoreceptors convert light signals into cellular cues that ultimately control gene expression during photomorphogenesis.[1−3] In vitro, phytochromes are easily recognized by their photochromic properties. They reversibly convert between red and far-red absorbing forms, termed Pr and Pfr, upon illumination with red (Pr → Pfr) or far-red (Pfr → Pr) light.

Phytochromes possess a widely conserved photosensory core composed of the PHY (phytochrome-specific), PAS (Per/ARNT/Sim), and GAF (cGMP-phosphodiesterase/adenyl cyclase/FhlA) domains. The N-terminal PAS and GAF domains hold the chromophore, a covalently bound linear tetrapyrrole.[4,5] The effector domains, which deliver chemical output such as phosphorylation events, are located on the C-terminal side of the PHY domain. Thus, phytochromes must be able to transmit a conformational signal generated at the chromophore through the photosensory core to properly gate biochemical activity.

An overarching goal of phytochrome research has been to uncover the structural nature of this signaling.[4] The discovery of bacterial phytochromes in the 1990s[6−9] greatly accelerated this work and led to the publication of several crystal structures of truncated phytochromes.[10−17] Whereas these crystal models all represented dark states, a number of secondary structural elements, sequence motifs, and single amino acids were suggested to have important roles in photoconversion. For example, the "tongue"[13] or "arm"[12] feature, a protrusion which extends from the PHY domain back onto the chromophore-binding pocket and which contains a highly conserved PRxSF (proline-arginine-X-serine-phenylalanine) sequence motif, was postulated to be a key mediator of the structural signal; however, these static crystallographic snapshots have not revealed the actual nature of structural signaling.

Some bacterial phytochromes have inverted photocycles with Pfr as the resting state, but the meaning of this is not well understood. Is the inversion a matter of regulation, or is the entire activity pattern inverted? The nature of structural signaling in inverted and regular phytochromes should be

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clarified to understand this better. Moreover, several residues around the chromophores are widely conserved, as illustrated in Figure S1. Indeed, extensive mutagenesis work has identified key residues in the chromophore-binding pocket that give rise to spectral changes or alter dark reversion rates when substituted.[18−22] In some cases, a single mutation can completely abolish photochromicity. One such example is His290 of the *Deinococcus radiodurans* phytochrome, which when replaced by glutamine gives rise to a mutant with very weak spectral response to illumination.[19] This could mean that the signaling chain is disrupted, but it could also indicate that the residue regulates the Pr or Pfr photostates. Again, the structural mechanism of signaling should be identified. Spectroscopy in the visible region cannot readily do so because it inherently reports on the chromophore's local environment. A new experimental approach is needed.

We recently introduced difference X-ray solution scattering to directly probe the global structural rearrangement that accompanies the Pr-to-Pfr transition.[23] We concluded that photoconversion in the photosensory core of the *D. radiodurans* bacterial phytochrome gives rise to an opening of the homodimer in the Pfr state through a dramatic increase in the separation of the opposing PHY domains, illustrated in Figure 1. Thus, difference X-ray scattering applied to

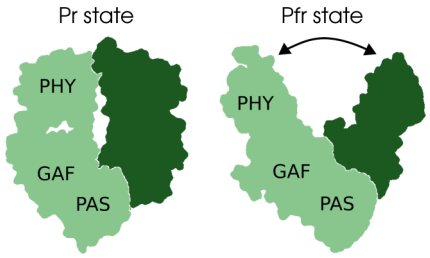

Figure 1. Light-induced opening of the photosensory core from *D. radiodurans* phytochrome, as previously proposed based on solution scattering experiments.[23]

phytochromes has the potential to report on structural rearrangements close to the biochemical output domains and can be used as a specific probe for signal propagation.

Difference scattering patterns result from conformational change and indicate its length scale. The signal is closely related to change in the molecule's distance distribution function.[24] In a qualitative way, intramolecular distances r are inversely related to the q scale by $r = 2\pi/q$, with $q = 4\pi/\lambda \cdot \sin\theta$, where 2θ is the scattering angle and λ is the X-ray wavelength. Whereas conventional small-angle X-ray scattering (SAXS) gives well-defined absolute scattering profiles for systems at equilibrium, the difference technique can robustly detect subtle rearrangements of protein structure and describe time-dependent structural processes (see Figure S2).[25,26] For proteins, direct structural interpretation is an inverse problem that requires testing a large number of well-chosen trial structures against experimental data;[27] however, if several comparable proteins give rise to similar difference X-ray patterns upon perturbation, it is reasonable to conclude that they undergo analogous conformational change.

Here we report difference X-ray scattering data on the photosensory cores of five bacterial phytochromes. Besides the previously published data on *D. radiodurans*, we also report data on two phytochromes (labeled P1 and P2) from the myxobacterium *Stigmatella aurantiaca* and the phytochromes Agp1 and Agp2 from *Agrobacterium tumefaciens*. Figure 2 shows X-ray scattering fingerprints of the Pr → Pfr conversion. Here,

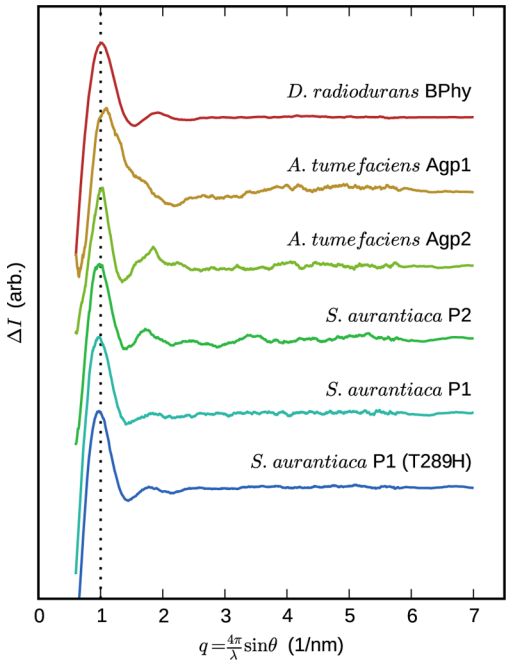

Figure 2. Solution X-ray difference scattering data, $\Delta I = I_{Pfr} - I_{Pr}$, where I_{Pfr} and I_{Pr} are scattered intensities after red or far-red illumination, respectively. These curves are not measured on an absolute scale and have therefore been scaled for comparison.

ΔI, plotted on the y axis, is the change in diffuse solution scattering intensity caused by red-light illumination ($\Delta I = I_{Pfr} - I_{Pr}$), and q is related to the scattering angle.[28] Note that the signal is resolved by measuring a large sample repeatedly, as detailed in the Supporting Information. We verified that radiation damage and the high concentration used did not affect the signal in the q range shown (Figure S2). The data show striking similarities between all investigated bacterial phytochrome homologues. While the difference signal between q = 1.5/nm and q = 3/nm varies from protein to protein, indicating minor differences, they all show a sharp feature at q = 1/nm. We conclude that these constructs undergo a similar quarternary-structural change upon illumination. In what follows, we discuss the implications of this for the understanding of structural signaling in bacterial phytochromes.

The first set of samples considered are the photosensory cores of *A. tumefaciens* Agp1 and Agp2, two phytochromes with opposing photochromic behavior. While Agp1 has a prototypical photocycle, meaning that its Pr state is thermally favored, Agp2 is a so-called bathy phytochrome that spontaneously converts to Pfr.[29] Importantly, Figure 2 shows that they give rise to very similar Pr → Pfr difference scattering signals, with some variation in the width and relative magnitudes of the peaks.

Since the discovery of these photosensors, the physiological, biochemical, and structural importance of the apparent inversion of Agp2 has not been fully understood.[29−32] Our results show that red light causes the same type of structural change in Agp2 as it does in prototypical phytochromes and imply that the difference between prototypical and bathy phytochromes is simply the direction of dark reversion. By analogy to our previous analysis of the *D. radiodurans*

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receptor,[23] this can be structurally interpreted as an open conformation in the dark-adapted Pfr state (see Figure 1). This interpretation, obtained from proteins in solution, could hold for other bathy phytochromes even if the only such crystal structure, that of the photosensory core from *Pseudomonas aeruginosa*, does not show an open arrangement of the dimer in the Pfr state.[12]

The similarity in structural change between Agp2 and prototypical phytochromes apparently contrasts kinase activity data previously reported by Karniol et al.,[29] according to which Agp1 and Agp2 have opposing biochemical functions. Their data show that Agp1 has higher autophosphorylation activity in the Pr state, while Agp2 is more active as Pfr. On the contrary, more recent data reported by Zienicke[33] show that Agp2 is instead more active in the Pr form and therefore behaves like most bacterial phytochromes do.[5] The latter result is in line with data on the bathy phytochrome from *P. aeruginosa* reported by Yang et al.[34] While our structural result cannot settle this question, we note that possible opposing Pr/Pfr activities would likely stem from events downstream of the structural rearrangement in the photosensory core. For example, the atypical output domain of Agp2, a HWE histidine kinase,[35] might interpret the structural signal differently from those of other bacterial phytochromes. Such a protein design, where the structural mechanism is conserved and the desired biological function is accomplished by attaching a different effector, would be in line with the idea that phytochromes and other photoreceptor proteins are highly modular entities.[36−38]

The second set of proteins are the phytochromes P1 and P2 from *S. aurantiaca*, which were discovered and characterized very recently.[22,39] While P2 is prototypical in its photochromicity, P1 displays an incomplete conversion to the absorption spectrum normally associated with the Pfr state (Figure 3a,b); however, the Pr → Pfr difference X-ray scattering

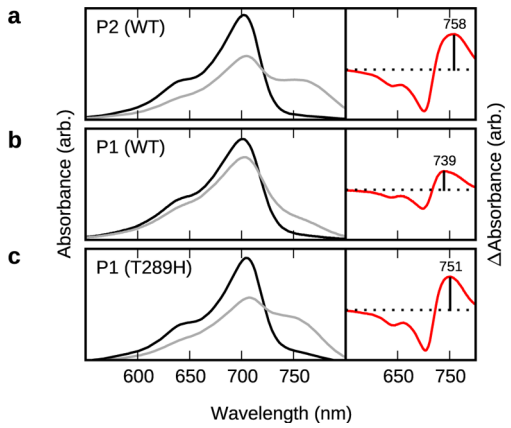

Figure 3. Steady-state absorption spectra of PAS-GAF-PHY fragments of the *S. aurantiaca* phytochromes 1 and 2 under far-red and red illumination (black and gray lines, respectively). Right-hand panel shows the difference between red illuminated and far-red illuminated samples.

in Figure 2 indicates that these proteins undergo structural change similarly to the *D. radiodurans* and *A. tumefaciens* phytochromes. Although only weakly photochromic, the P1 receptor produces the full structural rearrangement of a prototypical phytochrome.

The shape of the Pfr absorption spectrum of P1 shown in Figure 3b is reminiscent of mutated phytochromes where residues in the chromophore pocket are replaced.[18,19,21,40] In addition to a smaller change in absorption, the difference spectrum indicates a smaller red shift than for P2. In P1, the otherwise highly conserved His289 (corresponding to His290 in *D. radiodurans*, see Figure 4) is occupied by a threonine.

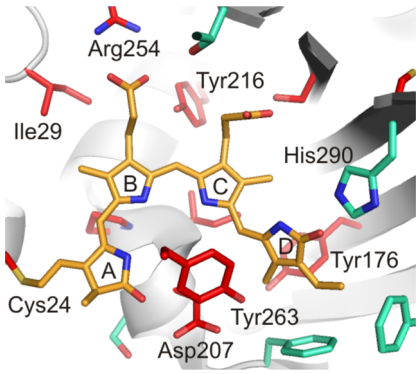

Figure 4. Conserved residues in the chromophore-binding pocket. The structure (PDB code 4Q0H[17]) and residue numbering is from *D. radiodurans*. The chromophore is shown in orange and conserved protein side chains are shown as red or cyan sticks according to their degree of conservation among bacterial phytochromes. Red indicates full conservation among the bacterial sequences aligned in Figure S1b, and cyan indicates partial conservation. For an interaction map, see Figure S1a.

Guided by this, we investigated the T289H mutant where the missing histidine has been reinserted. As seen in Figure 3c, this mutation restores much of the photochromicity of P1; however, the difference X-ray scattering (Figure 2) remains unaffected.

Two recent spectroscopic studies have investigated point mutations of this particular histidine and of nearby residues with hydrogen bonding ability.[39,41] It was found that substitution affects lifetimes and quantum yields during the early stages of the photocycle. This happens via altered hydrogen bonding with the chromophore and implies a regulatory role of the histidine. On the contrary it has been suggested based on photochromicity alone that removing the residue causes trapping in an intermediate state of the photocycle.[19] This would instead indicate that the residue has a key role in relaying the signal. Along the same lines, the histidine was recently assigned a crucial role in photoconversion via participation in proton transfers.[42] This conclusion was based on Raman spectroscopy of the chromophore. We note that while these studies are important for understanding the dynamics of the chromophore, they report only on its local environment.

Our results directly show that it is possible for a single mutation to have a dramatic effect on photochromicity without affecting large-scale structural photoconversion. The T289H substitution in *S. aurantiaca* P1 restores the far-red absorptivity of its Pfr state but has no measurable effect on global conformational change. With difference X-ray solution scattering, a distinction can be made between amino acid residues, which participate in signal propagation, and residues, which merely regulate photoconversion. Such regulation could, for instance, work by stabilizing a certain chromophore conformation, by tuning its absorbance at certain wavelengths, or perhaps by tuning the barrier to rotation of its rings. The distinction between signal-propagating and regulatory residues is not possible from chromophore absorption data alone.

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Previous analysis of the *D. radiodurans* PAS-GAF-PHY fragment showed that its scattering signal is consistent with a dramatic increase in PHY−PHY distance across the homodimer.[23] Because this structural change happens on a length scale close to the size of the molecule, few other conformational changes can explain the data. We therefore conclude that a similar change in the homodimer arrangement occurs in all of the bacterial photoreceptors considered here. The PHY domain is conserved to a high degree in plants, fungi, and bacteria, making it likely that this is a common, if not general, structural mechanism of signal transduction in phytochromes. We have also shown that the stabilization of the Pfr state in Agp2 and the chromatic adaptation of the Pfr spectrum in P1 from *S. aurantiaca* do not influence the light-induced conformational change. Thus, our results suggest that bacterial phytochromes have a conserved mechanism of signaling in the photosensory core and that adaptations to different cellular needs have evolved around this structural apparatus.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpclett.5b01629.

Experimental details and supporting figures. (PDF)

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Notes

The authors declare no competing financial interest.

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