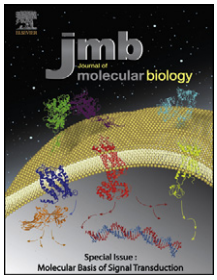

Structure of the Full-Length Bacteriophytochrome from the Plant Pathogen *Xanthomonas campestris* Provides Clues to its Long-Range Signaling Mechanism

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Abstract

Phytochromes constitute a major superfamily of light-sensing proteins that are reversibly photoconverted between a red-absorbing (Pr) and a far-red-absorbing (Pfr) state. Bacteriophytochromes (BphPs) are found among photosynthetic and non-photosynthetic bacteria, including pathogens. To date, several BphPs have been biophysically characterized. However, it is still not fully understood how structural changes are propagated from the photosensory module to the output module during the signal transduction event. Most phytochromes share a common architecture consisting of an N-terminal photosensor that includes the PAS2–GAF–PHY domain triad and a C-terminal variable output module. Here we present the crystal structure of the full-length BphP from the plant pathogen *Xanthomonas campestris* pv. *campestris* (*Xcc*BphP) bearing its photosensor and its complete output module, a PAS9 domain. In the crystals, the protein was found to be in the Pr state, whereas diffraction data together with resonance Raman spectroscopic and theoretical results indicate a *ZZZssa* and a *ZZEssa* chromophore configuration corresponding to a mixture of Pr and Meta-R state, the precursor of Pfr. The *Xcc*BphP quaternary assembly reveals a head-to-head dimer in which the output module contributes to the helical dimer interface. The photosensor, which is shown to be a bathy-like BphP, is influenced in its dark reactions by the output module. Our structural analyses suggest that the photoconversion between the Pr and Pfr states in the full-length *Xcc*BphP may involve changes in the relative positioning of the output module. This work contributes to understand the light-induced structural changes propagated from the photosensor to the output modules in phytochrome signaling.

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Introduction

Sunlight delivers vital radiant energy to our planet but it also provides spatial and temporal information sensed by organisms across all kingdoms. Biological photoreceptors detect light by sensing wavelength and intensity, transducing this information into cellular signaling pathways. For a long time, there had been no evidence for photoreceptors being functional in non-photosynthetic prokaryotes. However, over the last decade, several studies have shown that these proteins play many important roles including a role in bacterial infectious processes [1–3].

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Phytochromes are photosensors that can transduce light by a reversible photoconversion between two typical defined states: (i) red-absorbing (Pr) and (ii) far-red-absorbing (Pfr). These photoreceptors constitute a major family first described in plants, but also present in algae, fungi, cyanobacteria and other bacteria [4,5]. Prototypical phytochromes exhibit a Pr ground state and a dark conversion from Pfr to Pr. On the other side, the bathy-type phytochromes show a Pfr ground state and a Pr-to-Pfr dark conversion [6]. Most phytochromes share a common architecture consisting of an N-terminal photosensory module and a C-terminal variable output module. The photosensor detects the light signal, whereas the output module is responsible for transducing this information into a biological effect by means of a specific activity. The photosensor is composed of three consecutive domains: (i) Period/ARNT/Single-minded (PAS2), (ii) cGMP phosphodiesterase/adenylate cyclase/FhlA transcriptional activator (GAF), and (iii) phytochrome specific (PHY) [4]. Output modules are often composed of a single histidine kinase (HK) module; however, further variants include (i) an HK–response regulator pair, (ii) c-di-GMP cyclase/phosphodiesterase, or (iii) PAS domains, among others [7,8].

The photochemical properties of phytochromes reside in its open-chain tetrapyrrole (bilin), a bound chromophore that is buried within the GAF domain and that typically forms a covalent bond between the $C3^2$ atom of the side chain of ring *A* and a conserved cysteine residue of the phytochrome. The photochemistry takes place around the double bond between the bilin rings *C* and *D*, where an *E* to *Z* and *Z* to *E* isomerization of the C15 = C16 methine bridge rotates the *D* pyrrole ring defining the Pr (*ZZZssa*) and Pfr (*ZZEssa*) states, respectively [7]. The precise nature of the bilin differs within the phytochrome subfamilies: plant phytochromes incorporate phytochromobilin, cyanobacterial phytochromes phycocyanobilin, and bacteriophytochromes (BphPs) and fungal phytochromes biliverdin IXα (BV). All apo-phytochromes that are able to covalently bind the corresponding bilin perform this action autocatalytically by means of the bilin-lyase activity from the GAF domain [7].

To date, only few BphPs have been associated with specific biological responses [9,10]. *Xanthomonas campestris* pv. *campestris* (*Xcc*), a non-photosynthetic phytopathogenic bacterium that infects cruciferous plants, encodes a single BphP protein (*Xcc*BphP) that regulates its virulence[†]. Several BphP structures have been determined; however, none of them include a complete output module in addition to the photosensory module. Consequently, the structural determinants involved in photoreception and signaling between the photosensory and the output module remain elusive owing to the lack of a complete structural model. Here we provide novel data to address this issue by means of the full-length *Xcc*BphP crystal structure combined with spectroscopic, computational, and biochemical data.

Results

*Xcc*BphP is a bathy-like BphP

Sequence analysis on *Xcc*BphP revealed a conserved PAS2–GAF–PHY domain triad (Fig. 1) and a C-terminal PAS9 domain, according to a Pfam classification [11]. The PAS2–GAF–PHY triad represents the typical photosensory module from group-I canonical phytochromes found in bacteria, plants and fungi, whereas the PAS9 domain is the output module for this protein [4]. As the *Xcc*BphP PAS9 domain has no predicted enzymatic activity, it is likely to mediate protein–protein interactions in the signaling pathway [12]. Noteworthy, group-I phytochromes that contain a PAS domain immediately adjacent to the photosensor (alone or in combination with other domains) represent 48% of the Pfam database sequences to date. As expected, size exclusion chromatography (SEC) and zinc-induced fluorescence experiments [13] showed that BV binds both to the recombinant full-length *Xcc*BphP [14]—hereafter referred to as only *Xcc*BphP—and to a construct harboring the photosensory module alone (ΔPAS9) (Fig. S1).

Far-red irradiation (733 nm) leads to the photoconversion of both *Xcc*BphP and ΔPAS9 holoproteins into a pure Pr species, as indicated by the lack of the Pfr absorption band at 752 nm (Fig. 2a). Irradiation with red (630 nm) promotes the formation of the Pfr form, with a Pfr:Pr ratio of ~2:1 (Fig. 2b). In the absence of actinic light, dark conversion leads to the accumulation of the Pfr state, albeit to a different extent in the full-length protein and the truncated version (Fig. 2a). The ΔPAS9 construct exhibits a typical bathy-type BphP behavior, for which Pfr is the thermodynamically stable state, since a pure Pfr state is obtained after 21 h in the dark (Fig. 2b). In contrast to other representatives of the bathy phytochrome group [6], the presence of the PAS9 domain in *Xcc*BphP modulates the final Pfr:Pr ratio to ~6:1 (Fig. 2b), indicating thermal equilibrium between Pfr and Pr. We therefore denote *Xcc*BphP as a bathy-like phytochrome. The thermal Pfr/Pr equilibrium can be explained by the different quaternary arrangements in ΔPAS9 (monomer) and *Xcc*BphP (dimer, see Fig. 7), which might influence the chromophore binding pocket environment, and consequently the dark reversion thermodynamics [15].

To monitor the assembly process by UV–Vis absorption spectroscopy, both apoproteins were incubated in the dark with BV. *Xcc*BphP and ΔPAS9 assembled in less than 5 min as pure Pr forms. After 21 h in the dark, Pfr was the prevailing form in both

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