Minireview

Evolution of cyanobacterial and plant phytochromes

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Abstract Phytochromes are broadly distributed photochromic photoreceptors that are most sensitive in the red and far-red region of the visible spectrum. Three different bilins can be used as chromophores: plant phytochromes incorporate phytochromobilin, while phycocyanobilin serves as a chromophore of some cyanobacterial phytochromes, whereas all other phytochromes, including cyanobacterial orthologs incorporate biliverdin. During the evolution of plant and cyanobacterial phytochromes, the chromophore binding site has changed from a cysteine close to the N-terminus of the protein, the biliverdin attachment site, to a cysteine which lies within the so-called GAF domain and serves as phytochromobilin or phycocyanobilin attachment site. Since phylogenetic analyses imply that plant phytochromes are not direct successors of cyanobacterial phytochromes, chromophore exchange and the switch of the chromophore binding site has probably occurred at least twice in evolution. This may be regarded as an example for convergent evolution at the molecular level.

Keywords: Bilin; Chromophore binding site; Thioether; Phytochrome; Evolution; Phylogeny

1. Introduction

After rhodopsins, phytochromes were the second group of photoreceptors to be characterised biochemically. Due to their photochromic properties, phytochromes could be detected in plant tissue and extracts long before other plant photoreceptors were characterised [1]. As biliproteins, these photoreceptors are most sensitive in the red and far-red region of the visible spectrum [2]. Many developmental processes of plants, such as seed germination, flower induction, greening, leaf development, or shade avoidance, are controlled by phytochromes [2]. After the sequencing of the first plant phytochrome gene from oats [3], there are now about 80 full-length plant phytochrome sequences in public databases. Recent genome sequencing projects have revealed many phytochrome genes in bacteria and fungi, and around 50 full-length sequences from these organisms are presently found in public databases. Plant and bacterial phytochromes have a rather conserved N-terminal chromophore module with a domain structure as depicted in Fig. 1, and most of the bacterial phytochromes have a histidine-kinase module in the C-terminus. Molecular studies and database searches identified also quite a number of phytochrome-like proteins, which contain one or several GAF domains and various signal-transmitting domains or domains with unknown function [4–9]. Bacterial phytochromes and phytochrome-like proteins control diverse effects, such as chromatic adaptation [4], carotinoid synthesis [10,11], chlorophyll synthesis [12], circadian rhythm [6] or phototaxis [7]. Because the first prokaryotic phytochromes and phytochrome-like proteins were discovered in cyanobacteria [4,5,13–15], it was proposed that phytochrome evolution began in this group of prokaryotes [16,17]. An increasing number of sequences from other bacteria indicate, however, [10,18], that the origin of phytochromes must be set to the pre-cyanobacterial era [19].

2. Different chromophores and chromophore binding sites

It is long known that seed plant phytochromes carry an open chain tetrapyrrole (bilin), termed phytochromobilin (PΦB), as a chromophore [20,21] (see Fig. 1 for chromophore structure). This chromophore is probably used by all land plants, because PΦB is also used as a natural chromophore of a moss phytochrome [22]. Phytochrome from *Mesotaenium caldariorum*, a green alga, incorporates phycocyanobilin (PCB, see Fig. 1), which has a ring D ethyl instead of a vinyl side chain [23]. Typical cyanobacterial phytochromes also incorporate PCB as a natural chromophore, as shown for Cph1 of *Synechocystis* PCC6803 and CphA of *Calothrix* [24,25]. This bilin is produced by cyanobacteria as a component of phycobiliproteins, which serve as light-harvesting pigments for photosynthesis. A third bilin, bilirubin, might be used by PixJ, a cyanobacterial phytochrome-like protein with photochromic properties [26].

In oat phytochrome, a cysteine at position 322, termed position A in the present article (Fig. 1), has been identified as the chromophore attachment site [20,21]. This cysteine is conserved in all plant phytochromes known to date, and in most but not all cyanobacterial phytochromes and phytochrome-like proteins. It is generally accepted that the position A cysteine, if present, serves as chromophore attachment site. In some other cyanobacterial phytochromes and in all non-cyanobacterial non-plant phytochromes known to date, this cysteine residue is lacking [10,16,27]. After the discovery of these phytochromes it turned out that proteobacteria and

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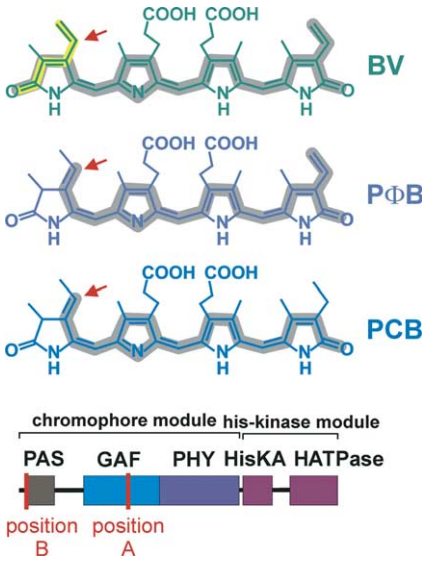

Fig. 1. Phytochrome chromophores biliverdin (BV), phytochromobilin (PΦB) and phycocyanobilin (PCB); domain arrangement of a typical bacterial phytochrome (lower panel). The C-atom of the chromophore that is coupled to the cysteine residue is labelled with a red arrow; the adjacent double bond becomes reduced during the coupling reaction. In BV, this double bond is part of a short π-electron system (highlighted in yellow), but not part of the long π-electron system (highlighted in grey). Since the spectral properties are determined by the long π-electron system, covalent attachment does not alter the absorbance maximum of BV [29,31]. In PCB and PΦB, this double bond is part of the long π-electron system (highlighted in grey). In this case, the chromophore absorbance maximum shifts to lower wavelengths upon covalent attachment [37]. The cartoon of the lower panel depicts the domain arrangement of a typical bacterial phytochrome. Plant phytochromes have a similar chromophore module, but contain an additional N-terminal extension. The PAS domain in the N-terminus is only recognized in few bacterial phytochromes. Between the chromophore module and the histidine-kinase-like domain of plant phytochromes there is a ca. 300 amino acid region containing two PAS domains. Phytochromes have a cysteine either at position A or at position B. The domain organization of phytochrome-like proteins differs from the above scheme.

other prokaryotes do not use PCB or PΦB as a chromophore, but rather biliverdin (BV), which is a precursor in the PCB and PΦB biosynthesis pathway [28]. The lack of the position A cysteine correlates with the use of BV; all tested phytochromes of this group covalently bound BV in vitro [12,25,28,29]. In the cyanobacterium *Calothrix*, which carries one phytochrome with and one without a position A cysteine (CphA and CphB), the former incorporates PCB in vivo, the latter BV [25].

For *Deinococcus* phytochrome, it was originally proposed that the chromophore binds to His260 [10]. This histidine lies immediately next to the position A amino acid on the C-terminal side, and is conserved in all phytochromes known to date. Evidence for the role of His260 is based on the mutant His260Ala, which did not incorporate any chromophore, and mass spectrometry. As it was not known at that time that BV serves as natural chromophore of *Deinococcus* phytochrome, these analyses were performed with PCB, which does not form a covalent link with other phytochromes of this type. Indirect evidence was presented that a histidine could also be the BV attachment site of *Calothrix* CphB [25]. However, blocking studies and site-directed mutagenesis with *Agrobacterium* phytochrome Agp1 showed that a cysteine residue close to the N-terminus, Cys20, is required for covalent BV attachment [29]. The homologous position is termed position B here (Fig. 1). MALDI-TOF and ion-trap mass spectrometry measurements showed that BV is indeed coupled to Cys20 [30]. Assembly studies performed with synthetic BV-derivatives showed that the ring A vinyl side chain of BV is important for covalent binding [31]. It seems therefore that the vinyl A side chain of BV forms a covalent thioether bond with the cysteine residue.

Quite interestingly, the position B cysteine is conserved in all those phytochromes that are lacking the position A cysteine, i.e., those phytochromes with a proposed BV chromophore. Therefore, the position B cysteine most likely serves as a chromophore attachment site in all BV-binding phytochromes, not only in *Agrobacterium* Agp1. New results on *Deinococcus* phytochrome support the role of the position B cysteine, Cys24 in the *Deinococcus* phytochrome. This amino acid was replaced by different other amino acids. All mutant proteins incorporated BV in a non-covalent manner. Mutants in which His260 was replaced by glutamate still bound the chromophore covalently (J.R. Wagner and R.D. Vierstra, personal communication). In *Pseudomonas aeruginosa* phytochrome the chromophore is probably bound to a cysteine residue in the N-terminus of the protein (N. Frankenberg, personal communication).

The distribution of bilin synthases among organisms implies that BV is the more ancient type of chromophore. Biliverdin is synthesized from heme in a one-step reaction by the enzyme heme oxygenase (HO). Plant phytochromobilin and cyanobacterial PCB are formed from BV by either PΦB synthase [32] or PCB synthase, PcyA, [33], respectively. The biosynthesis of PCB in *Mesotaenium* differs from cyanobacteria, since the conversion of BV into PCB proceeds via PΦB and involves another yet unidentified enzyme [23]. Heme oxygenase is present in most organisms, whereas PCB- and PΦB synthases are restricted to plants, cyanobacteria and red algae, as shown by [32] and recent BLAST searches. Also, the BV-binding cysteine at position B must be regarded as the more ancient chromophore binding site. The chromophore switch could reflect an adaptation to the chlorophyll environment. Whereas BV adducts have an absorbance maximum of ca. 700 nm [28,29,34], PCB and PΦB adducts have absorbance maxima around 655 and 665 nm, respectively, [21,35]. These spectral properties match well with the absorbance properties of chlorophyll a in vivo. In plants, phytochromes control many effects that are related to chlorophyll synthesis and chlorophyll screening.

Phytochrome chromophore assembly has been analysed for PΦB and PCB binding phytochromes [36,37], but the detailed reaction mechanism is as yet unknown. The switch of the chromophore binding site can give a deeper insight into this mechanism. Most likely, all chromophores are bound by a thioether bond formed between the $C3^1$ atom and the sulphur residue of the cysteine, but the double bond which becomes reduced during the chemical reaction has different properties: in BV, it lies between $C3^2$ and $C3^1$ and is part of a short π electron system with 3 double bonds, whereas in PΦB and PCB, it lies between $C3^1$ and C3 and is part of the long π electron system with 11 and 10 double bonds, respectively (see Fig. 1). This difference is obviously compensated by the differentially located reaction partner. Position A and position B amino acids must be close together within the tertiary structure of phytochromes, but the corresponding reactive sites must be spatially separate to explain the different reaction specificity.

Calothrix CphB and *Agrobacterium* Agp1 incorporate PΦB and PCB in a non-covalent manner, but when a cysteine residue is introduced at position A, the covalent reaction with the ethylidene side chain becomes possible [27,31]. With BV, the situation is the other way round: this chromophore interacts with plant [36] and cyanobacterial phytochromes with a position A cysteine [38], but is not covalently bound to these proteins.

3. Evolution of PCB and PΦB binding phytochromes

It has often been proposed that plant phytochromes were inherited from the cyanobacterial endosymbiont that gave rise to plant plastids [16,17,19,39,40]. This view was supported by the discovery of BV-binding phytochromes. However, cyanobacterial and plant phytochrome sequences are not that homologous to each other [19,40]. Our own phylogenetic analyses, which included novel phytochrome sequences and which were performed with protein sequences of either the chromophore module (Fig. 2) or the histidine kinase module (Fig. 3) also showed that plant and cyanobacterial sequences are rather distantly related. The C-terminal histidine kinase moiety is inherent to most bacterial (see Fig. 1) and fungal phytochromes, and plant phytochromes bear a histidine-kinase-related module in their C-terminus. Selection pressure on this part of the protein is probably different from that on the N-terminal chromophore module. Therefore, the histidine kinase module can be used for an independent assay of protein relationship. In both phylogenetic trees, plant, fungal and cyanobacterial phytochromes formed separate branches or at least closely related groups indicating that members of each group evolved from a single progenitor (Figs. 2 and 3). Both kinds of phylogenetic analyses were also performed with smaller numbers of protein sequences (e.g., 3 fungal, 8 plant, 5 cyanobacterial and 14 other bacterial sequences) or other alignment parameters (lower gap penalties). The default matrix for the amino acid replacement model of the PROTDIST [41] program is the Jones-Thornton-Taylor matrix [42]. In comparative runs, the older Dayhoff PAM matrix [43] was also used. In neither case did the plant branch originate from a position close to the cyanobacterial group, i.e., proteobacterial species always grouped between cyanobacteria and plants, although the insertion points varied from case to case, a finding which is expected with the rather low bootstrap values in the core of the phylogenetic trees. Since a rather big distance between plants and cyanobacteria was found in analyses performed with two independent regions of the protein, one from the N-terminus and one from the C-terminus, it is very unlikely that a direct relationship between both groups has been overseen.

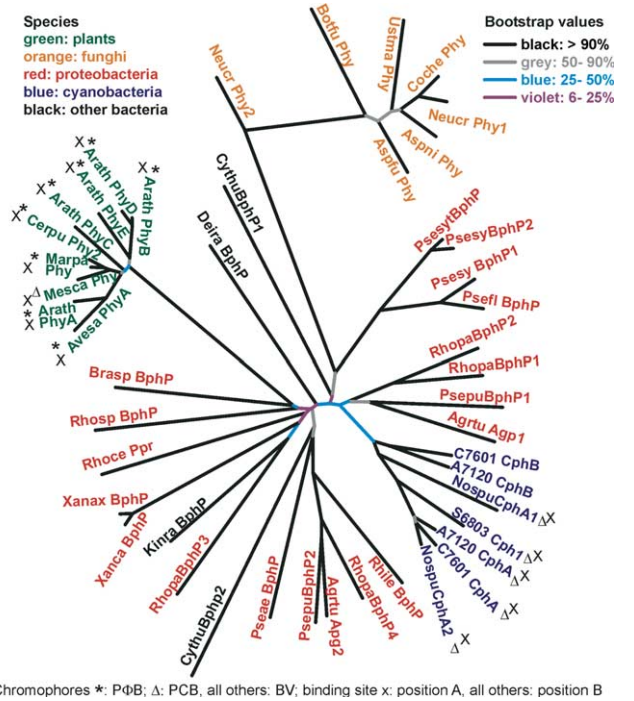

Fig. 2. Phylogenetic tree of phytochromes, constructed from the N-terminal chromophore module. Protein sequences were aligned using ClustalX [47], version 1.83, with the "gap opening" parameter set to 40. Regions with gaps were removed. The phylogenetic tree was constructed with the PHYLIP program package version 3.6 [41] using the SEQBOOT, PROTDIST, FITCH, CONSENSE and DRAWTREE programs with default parameters. For bootstrapping, 100 datasets of sequences were generated. The abbreviation of most species names is given in [30].

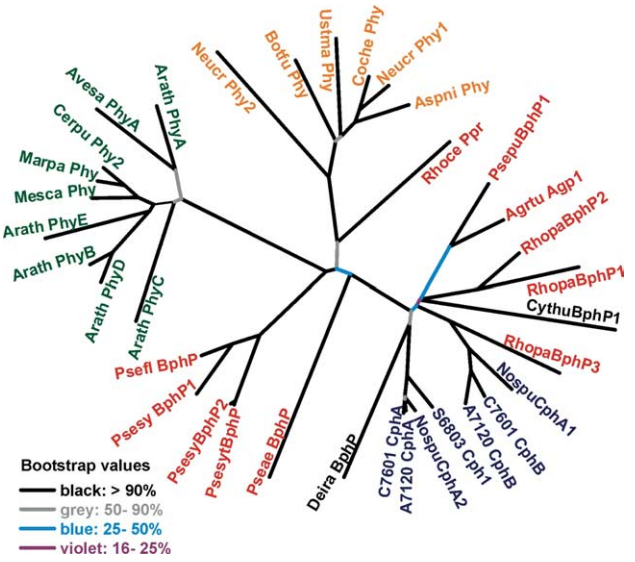

Fig. 3. Phylogenetic tree of phytochromes, constructed from the C-terminal histidine-kinase module or histidine-kinase-related region. Phytochromes with an unrelated histidine-kinase (e.g., AgrtuAgp2, CythuBphP2, AspfuPhy) or with a completely divergent C-terminus (BraspBphP) were not included.

Thus, plant phytochromes were most likely not inherited from cyanobacteria but were already present in the cell when the cyanobacterial endosymbiont gave rise to plant plastids. Quite interestingly, the same conclusion can be drawn for HOs. In phylogenetic analyses, plant HOs appeared closer related to animal and some proteobacterial HOs than to cyanobacterial orthologs [44,45].

The second enzyme of chromophore synthesis in plants, PΦB synthase, stems probably from cyanobacteria, because there are no homologs in other prokaryotes. However, PΦB synthase is closer related to phycoerythrobilin synthase, PebB, than to PCB synthase [33], and is thus no direct successor of the latter.

The biological role of cyanobacterial and plant phytochromes is quite divergent, supporting the evolutionary distance between both groups of photoreceptors. Phytochrome regulation affects almost any step in plant development, whereas only subtle effects have been reported for Cph1 and Cph2 of the cyanobacterium *Synechocystis* PCC 6803 [46]. Biological effects of phytochromes in other cyanobacteria are as yet unknown, and many observable light-dependent processes in cyanobacteria are rather controlled by phytochrome-like proteins RcaE [4], PlpA [5], CikA [6], or PixJ [7], which have no direct plant homologs.

All observations together channel into the following possible scenario for the evolution of PCB or PΦB binding phytochromes:

(i) Cyanobacteria invented water-oxidation, chlorophyll a synthesis, phycobiliprotein antenna pigments, and BV-reducing enzymes such as PcyA, PebA and PebB, but neither phytochrome nor HO. These were inherited from a proteobacterial predecessor. Gene duplication gave then two phytochromes. The chromophore binding site of one of these switched from position B to position A, thereby preferring PCB over BV. Both types of phytochromes can still be found in some cyanobacteria [16,27]. Since only few functions of cyanobacterial phytochromes are known, the reason for this switch is speculative. Either, phytochromes were/are used as regulators of bilin biosynthesis, where PCB is measured by phytochromes with a position A cysteine and BV by phytochromes with a position B cysteine. Or the above mentioned spectral shift towards shorter wavelengths favoured the evolution of phytochromes with a position A cysteine, once the corresponding bilin was available. The phytochrome-like proteins RcaE, PixJ, CikA, PlpA and others probably evolved in cyanobacteria after combining phytochrome-derived domains with other protein domains.

(ii) The eukaryotic predecessor of plants contained phytochrome before a cyanobacterium entered the cell as photosynthesis endosymbiont. This phytochrome must have used BV as a chromophore, synthesised by HO which was also already present in the cell. After cyanobacterial endosymbiosis, enzymes for the reduction of BV ring A became available. Of these, plants maintained only the phycoerythrobilin synthase PebB, which evolved into PΦB synthase, others like PcyA were lost. The phytochromes of the cyanobacterial endosymbiont were also lost, probably because these were of no use within the chloroplast, where they originally must have been located. With the availability of PΦB, the chromophore binding site of phytochrome switched from position B to position A. By this step, plants adapted light regulation to the chlorophyll a – rich environment. According to the phylogenetic analyses shown in Figs. 2 and 3, the evolution into different types of plant phytochromes has occurred much later in evolution, probably at a time when BV binding phytochromes were already lost. Other events that formed the typical more or less universal plant phytochrome domain structure must have preceded this differentiation, e.g., during early plant evolution.

The above scenario can be regarded as an example for convergent evolution at the molecular level. Based on mutant analyses performed with two different phytochromes it is clear that the switch of chromophore selectivity from BV to PCB/PΦB requires only two events: the amino acid at position A must be replaced by a cysteine, and the cysteine at position B replaced by another amino acid. Both events can have occurred one after the other. It is not hard to imagine that this step has occurred several times in the evolution.

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