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Red-shifted red/green-type cyanobacteriochrome AM1_1870g3 from the chlorophyll *d*-bearing cyanobacterium *Acaryochloris marina*

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ABSTRACT

Cyanobacteriochromes (CBCRs) are diverse photoreceptors that are found only from cyanobacteria and cover wide range of light qualities. CBCRs are divided into two types regarding the chromophore species they contain: phycocyanobilin (PCB) and phycoviolobilin. Red/green-type CBCRs are widely distributed subfamily among the PCB-binding CBCRs and photoconvert between a red-absorbing thermostable form and a green-absorbing metastable form. Our recent study discovered that a red/green-type CBCR, AM1_1557g2, from a cyanobacterium *Acaryochloris marina* covalently binds not only PCB but also biliverdin (BV). BV-binding AM1_1557g2 photoconverts between a far-red absorbing form and an orange-absorbing form. We report, herein, that another red/green-type CBCR, AM1_1870g3, from the cyanobacterium *A. marina* also bound both PCB and BV. PCB- and BV-binding ones showed red/green and far-red/orange reversible photoconversions, respectively. Unexpectedly, absorbing wavelengths are 10–20 nm red-shifted compared with those of AM1_1557g2. These red-shifted characteristics may be useful for optogenetic light switches that work in various organisms.

1. Introduction

Cyanobacteriochromes (CBCRs) are recently identified linear tetrapyrrole-binding photoreceptor family that is found only in cyanobacteria to date and is diverse in their binding chromophores and their sensing light qualities [1]. CBCRs are distant relative of red/far-red light-sensing phytochromes and involved in some photo-acclimation processes such as phototaxis [2–7], chromatic acclimation of photosynthetic antennae [8] and light-dependent cell aggregation [9]. CBCRs need only GAF (cGMP-phosphodiesterase/adenylate cyclase/FhlA) domain to ligate chromophores, whereas the phytochromes need a large unit composed of PAS (Per/Arnt/Sim), GAF and PHY (phytochrome-specific) domains for chromophore ligation. CBCRs are divided into two subfamilies regarding the binding chromophore they contain: phycocyanobilin (PCB) and phycoviolobilin. PCB-binding CBCRs sense relatively long wavelength light of blue-to-red region [10–14], whereas phycoviolobilin-binding CBCRs sense rather short wavelength light of ultraviolet-to-green region [15–20].

Among the PCB-binding CBCRs, red/green-type CBCRs are widely distributed and considerably analyzed so far [10,14,21]. The red/green-type CBCRs covalently bind PCB and reversibly photoconvert between a red-absorbing thermostable form (Pr) and a green-absorbing metastable form (Pg). The red-absorbing form is quite similar to that of the phytochromes, whereas the green-absorbing form is clearly different from the far-red absorbing form of the phytochromes. Although structural and spectral studies suggest that the unique blue-shift of the Pg form is due to ring D twist and/or chromophore hydration, most of their color tuning mechanisms remain still unclear [22–27].

Recently, we identified that a red/green-type CBCR AM1_1557g2 from the chlorophyll *d*-bearing *Acaryochloris marina* covalently binds not only PCB but also BV [28]. BV-binding AM1_1557g2 shows reversible photoconversion between a far-red absorbing thermostable form (Pfr) and an orange-absorbing metastable form (Po). The Pfr form fluoresces with a maximum at 730 nm. Because *A. marina* mainly utilizes chlorophyll *d* as a photosynthetic pigment that is red-shifted compared with chlorophyll *a*, the BV-binding ability of AM1_1557g2 may be a physiologically relevant feature of *A. marina*.

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In this study, we identified another red/green-type CBCR AM1_1870g3 from *A. marina* that also covalently binds both PCB and BV. Both PCB- and BV-binding ones absorb 10–20 nm red-shifted light qualities compared with those of the other red/green-type CBCRs. In this context, potentials of this red-shifted CBCR as the optogenetic switch are discussed.

2. Materials and methods

2.1. In silico characterization of AM1_1870

The domain composition of AM1_1870 was predicted using SMART program on the web site (http://smart.embl-heidelberg.de/) [29]. Alignment and phylogenetic clustering of CBCR and phytochrome GAF domains were performed by CLUSTAL_X [30]. The phylogenetic tree was drawn by Dendroscope [31].

2.2. Plasmid construction

The nucleotide sequence of AM1_1870g3 was cloned into pET28a (Novagen) using the In-Fusion HD Cloning kit (TaKaRa). The DNA fragment corresponding to *AM1_1870g3* was PCR amplified using the synthetic primers 5′-CGCGGCAGCCATATGGTCCAGACCCTCAATACC-3′ (forward primer) and 5′-CTCGAATTCGGATCCTCAAACGGCGAGCTGTTGCGC-3′ (reverse primer), genomic DNA from *A. marina* MBIC11017, and PrimeSTAR Max DNA polymerase. pET28a was PCR amplified using the synthetic primers 5′-CATATGGCTGCCGCGCGG-3′ (forward primer) and 5′-GGATCCGAATTCGAGCTC-3′ (reverse primer), pET28a, and PrimeSTAR Max DNA polymerase. A plasmid expressing AM1_1870g3 (pET28a_AM1_1870g3) was then constructed with the TaKaRa in-fusion system reagents. The sequence of the gene encoding AM1_1870g3 was verified by DNA sequencing.

2.3. Expression and purification of His-tagged AM1_1870g3

Escherichia coli C41 (Novagen) carrying pKT270 or pKT271 [32] was used for AM1_1870g3 expression. Each culture was incubated at 37 °C for 2.5 h in 1 L of Luria-Bertani medium, 20 μg ml^{-1} kanamycin, and 20 μg ml^{-1} chloramphenicol, followed by addition of isopropyl-thio-β-D-galactopyranoside (final concentration, 0.1 mM). Cells were then cultured at 18 °C overnight, after which they were harvested by centrifugation, frozen at −80 °C, thawed at 4 °C, and suspended in 50 ml of Buffer A (20 mM HEPES-NaOH, pH 7.5, 100 mM NaCl, 10% (w/v) glycerol). Cells were disrupted by three passages through an Emulsiflex C5 high-pressure homogenizer at 12,000 psi (Avestin). The cell extract was then centrifuged at 109,200× *g* for 30 min at 4 °C. Each supernatant was individually passed through a nickel-affinity His-trap chelating column (GE Healthcare). After washing the column with Buffer A containing 30 mM imidazole, the binding proteins were eluted using a step gradient of 50, 100, and 200 mM imidazole in Buffer A. His-tagged proteins were mostly eluted in the 200-mM imidazole fraction, which was studied after removal of imidazole by dialysis against Buffer A.

2.4. SDS-PAGE and Zn-induced fluorescence assay

Proteins in 2% (w/v) lithium dodecylsulfate, 60 mM DTT, 60 mM Tris–HCl, pH 8.0 were subjected to SDS-PAGE (15% (w/v) acrylamide), followed by staining with Coomassie Brilliant Blue (CBB) R-250. For the Zn-induced fluorescence assay, after SDS-PAGE, the gel was soaked in 20 μM zinc acetate at room temperature for 30 min [33]. Then, fluorescence was visualized through a 605 nm filter upon excitation at 532 nm (FMBIO II; Takara).

2.5. Spectroscopy

Ultraviolet and visible absorption spectra of the proteins were recorded using a Shimadzu UV-2600 spectrophotometer at room temperature. Monochromic light of various wavelengths was generated using a variable wavelength light source (Opto-Spectrum Generator, Hamamatsu Photonics, Inc.). After denaturing the proteins in 8 M urea, pH 2.0, their absorption spectra were recorded. Then, the protein samples were irradiated with white light for 3 min, and absorption spectra were again recorded.

3. Results and discussion

3.1. Sequence characteristics of AM1_1870g3

AM1_1870 is a multi-domain two-component signal-transduction protein of 1514 amino acid residues. AM1_1870 is composed of four GAF domains, one His kinase (HK) domain, two response regulator (RR) domains and one phospho-accepting (HPT) domain (Fig. 1A). Third GAF domain (AM1_1870g3, 512-668 residues) belongs to the red/green-type CBCR GAF domain according to our clustering analysis, whereas first and fourth GAF domains belong to phytochrome-type and blue/green-type, respectively (Fig. 1B). These three GAF domains retain residues that are important for chromophore ligation and photoconversion. In this context, AM1_1870 is a quite unusual protein that contains three distinctive light sensing domains within one protein.

3.2. Photoconversion of AM1_1870g3-PCB and AM1_1870g3-BV

AM1_1870g3 expressed in a PCB-producing *E. coli* was purified into near homogeneity (Fig. 2A, CBB). PCB covalently bound to AM1_1870g3 judging from Zn-dependent fluorescence assay (Fig. 2A, +Zn). AM1_1870g3-PCB showed reversible

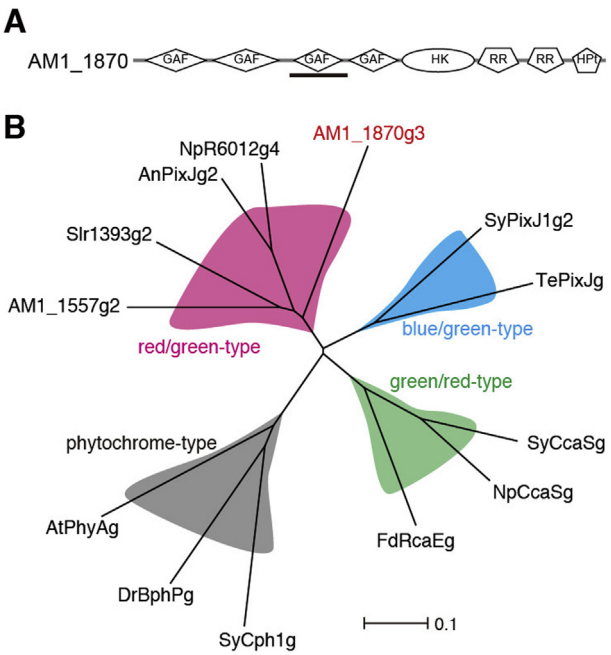

Fig. 1. AM1_1870. (A) Domain architecture of AM1_1870 according to a SMART motif analysis. (B) Cluster analysis of red/green-, green/red- and blue/green-type CBCR and phytochrome-type GAF domains. The position of AM1_1870g3 on the tree was highlighted in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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