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^{1}H, ^{13}C, and ^{15}N Chemical Shift Assignments of Cyanobacteriochrome NpR6012g4 in the Green-Absorbing Photoproduct State

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Abstract

Cyanobacteriochromes (CBCRs) are cyanobacterial photosensory proteins with a tetrapyrrole (bilin) chromophore that belong to the phytochrome superfamily. Like phytochromes, CBCRs photoconvert between two photostates with distinct spectral properties. NpR6012g4 from *Nostoc punctiforme* is a model system for widespread CBCRs with conserved red/green photocycles. Atomic-level structural information for the photoproduct state in this subfamily is not known. Here, we report NMR backbone chemical shift assignments of the light-activated state of NpR6012g4 (BMRB no. 26577) as a first step toward determining its atomic resolution structure.

Keywords

Cyanobacteriochrome; CBCR; Photoreceptor; Phytochrome; Cyanobacteria; phycocyanobilin; NMR

Biological Context

Phytochromes are photosensory proteins utilizing covalently attached linear tetrapyrrole (bilin) chromophores that photoconvert between red- and far-red-absorbing states (Auldridge & Forest, 2011). In cyanobacteria, related cyanobacteriochrome (CBCR) sensors provide complete coverage of the spectrum from near-ultraviolet to red (Ikeuchi & Ishizuka, 2008). Phytochromes and CBCRs share a bilin-binding GAF domain with slight structural differences. One widespread CBCR subfamily typically exhibits red/green photocycles in which the red-absorbing dark state interconverts with a green-absorbing photoproduct (Rockwell et al, 2012). Such CBCRs are known to regulate phototaxis (Campbell et al, 2015; Enomoto et al, 2015). Structural information for these red/green CBCRs is limited to a crystal structure for AnPixJg2 in the red-absorbing dark state (Narikawa et al, 2013). The blue-shifted absorption of the photoproduct state arises due to a trapped-twist mechanism in which conserved aromatic residues are essential (Rockwell et al, 2014; Rockwell et al,

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2015b), but there is no information about changes in the protein structure upon photoconversion.

NpR6012g4 from *Nostoc punctiforme* has been extensively studied as a model system for such red/green CBCRs (Kim et al, 2012; Rockwell et al, 2014; Rockwell et al, 2012; Rockwell et al, 2015b). The phycocyanobilin (PCB) chromophore is covalently attached to a conserved cysteine (Cys589) at the bilin A-ring and adopts a C15-*Z*, *anti* configuration with all 4 NH moieties protonated (Rockwell et al, 2015b). Photoconversion yields the C15-*E,anti* chromophore and places the photoactive D-ring in a hydrophobic environment (Rockwell et al, 2015a). Biological signaling is thought to arise via propagation of these structural changes to adjacent domains, but atomic resolution structures of NpR6012g4 in both states are needed to elucidate such changes. Here, we report NMR resonance assignments for the green-absorbing light-activated state of NpR6012g4 as a first step toward determining its atomic resolution structure.

Methods and Experiments

Expression and Purification of NpR6012g4.

The protein sample in this study consists of 180 native residues (M583-G762, Fig. 1) after removal of a C-terminal intein-CBD tag used for affinity purification (Kim et al, 2012). NpR6012g4 was expressed in BL21-AI cells (Invitrogen) grown in M9 minimal media supplemented with ALA (100 μM), ^{15}N-labeled ammonium chloride, and/or ^{13}C-labeled glucose (Cambridge Isotopes) using a published system for induction of protein expression and chromophore biosynthesis (Gambetta & Lagarias, 2001). Affinity purification of NpR6012g4 using a chitin column (NEB) followed our previous procedure (Kim et al, 2012; Rockwell et al, 2012; Rockwell et al, 2015a; Rockwell et al, 2015b). Peak eluted fractions were pooled for overnight dialysis into 10 mM sodium phosphate (pH 7.4) supplemented with 1 mM EDTA to remove residual metal ions followed by final overnight dialysis into 10 mM sodium phosphate (pH 7.4). The protein was concentrated to approximately 0.7 mM, and D_2O was added to 7% (v/v). Dark reversion of the metastable green-absorbing state under these conditions was < 10% after 24 hours at 298 K as reported previously (Rockwell et al, 2015b). All subsequent manipulations were performed on samples kept in darkness.

NMR spectroscopy.

NMR experiments were recorded using Bruker Advance 600 MHz spectrometer equipped with a triple resonance cryogenic probe. All experiments were performed in darkness to prevent light-induced photoconversion leading to sample inhomogeneity. Multi-dimensional ^{1}H-^{15}N HSQC, HNCO, HNCACO, HNCA, HNCACB, CBCACONH, HBHACONH, ^{1}H-^{15}N TOCSY-HSQC (mixing time of 60 ms), and ^{1}H-^{15}N-NOESY-HSQC (mixing time of 120 ms) spectra were obtained at 298 K. NMR data were processed using NMRPipe. Backbone assignments were made using the SPARKY software package (www.cgl.ucsf.edu/home/sparky).

Assignments and Data Deposition

Two-dimensional NMR spectra of the NpR6012g4 photoproduct, ^{15}N-^{1}H HSQC (Fig. 2a) and constant-time ^{13}C-^{1}H HSQC (Fig. 2b) are presented to illustrate representative NMR assignments for backbone and side-chain methyl resonances, respectively. The spectra exhibit well-resolved peaks with uniform intensities, indicative of a stably folded protein structure. Several amide resonances exhibit noteworthy downfield shifts, including E656, N677, A716, and N720. These residues are predicted by the AnPixJ crystal structure to lie close to aromatic rings (W655, W726, Y718, and W643, respectively), which may explain the downfield ring current shifts. Chemical shift assignments were determined by analysis of triple resonance NMR experiments performed with ^{15}N or ^{15}N/^{13}C-labeled protein samples (residues 583-762). All protein NMR resonances were assigned except for resonances in the N-terminal region (residues 583-598), corresponding to an -helix (1) in the AnPixJ crystal structure (Narikawa et al, 2013). More than 90% of the backbone resonances (^{1}HN, ^{15}N, ^{13}C, ^{13}C, and ^{13}CO) and 80% of the methyl side-chain resonances were assigned. A complete list of chemical shifts of ^{1}H, ^{15}N, ^{13}C resonances assigned to the NpR6012g4 photoproduct have been deposited in the BioMagResBank repository under the BMRB accession number, 26577.

The NMR-derived protein secondary structure of the NpR6012g4 photoproduct was calculated on the basis of NMR chemical shift index (Wishart et al, 1992) using the software, TALOS+ (Fig. 1). The chemical shift index calculated for each residue is shown above the calculated secondary structural elements in Fig. 1b. Secondary chemical shifts for ^{13}C versus residue number are shown in Fig. 1c. The NMR-derived secondary structural elements of the NpR6012g4 photoproduct are assigned as follows: 2: residues 600-616; 1: residues 620-626; 2: residues 635-640; 3A: residues 645-647; 3A: residues 659-662; 3B: residues 667-669; 4: residues 673-676; 4A: residues 679-681; 4B: residues 688-694; 5: residues 699-707; 6: residues 710-719; 5: residues 728-753. The numbering of helices and strands here is the same as that used previously for the AnPixJ crystal structure (Narikawa et al, 2013). The overall topology and secondary structure of the NpR6012g4 photoproduct is quite similar to that of the NpR6012g4 dark-state (Fig. 1a). The only light-dependent difference in secondary structure of NpR6012g4 is detected for residues 654-656 (marked by a blue box in Fig. 1b), which form a short -strand in the dark-state rather than the random coil structure seen in the photoproduct. Interestingly, the corresponding residues in AnPixJ also form a -strand in the dark-state crystal structure (residues 88-90) (Narikawa et al, 2013), and a light-induced structural transition involving the third strand of AnPixJ has been proposed (Velazquez et al, 2013). We hypothesize that light-induced unfolding of the third -strand might be a general feature of red/green CBCR photoconversion. Future NMR studies will determine the atomic-level structure of the green-absorbing NpR6012g4 photoproduct to more fully elucidate mechanisms of spectral tuning and target recognition.

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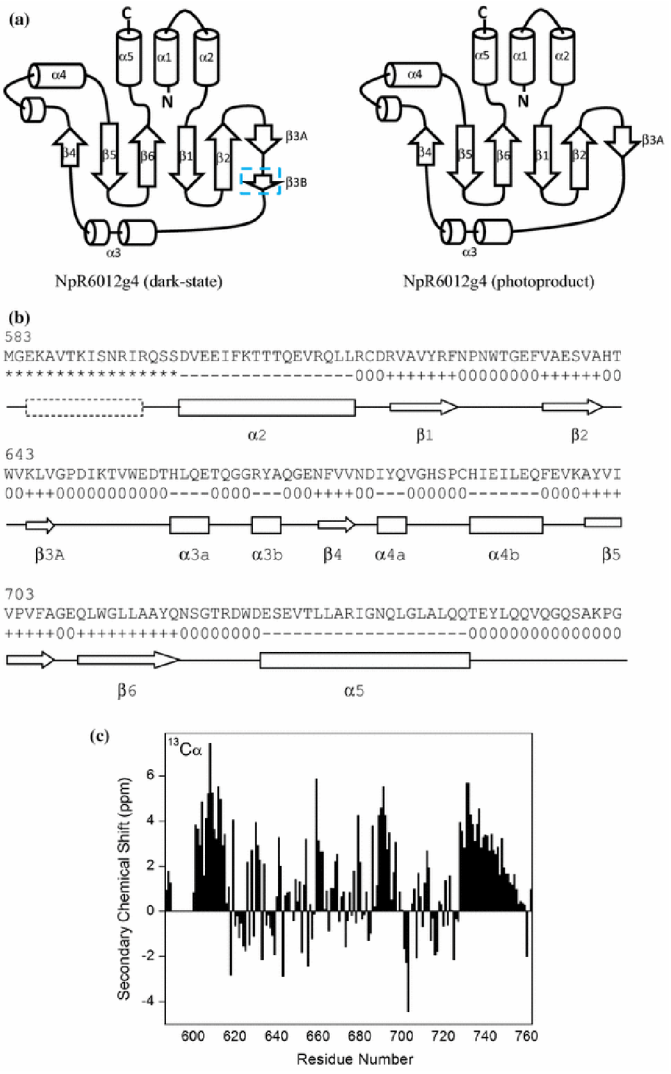

Figure 1.

Amino acid sequence and topologies of dark- and lit-states of NpR6012g4. a) Topology diagrams show NpR6012g4 in the red-absorbing dark state (left panel) determined in a companion paper and green-absorbing light-activated state (right panel) determined in this study. The blue dashed box highlights a strand (3B) at residues 654-657 in the dark-state that becomes random coil in the photoproduct. b) Secondary structural elements for NpR6012g4 in the photoproduct state are derived from analysis of chemical shift index (Wishart et al, 1992) and sequential NOE patterns. The chemical shift index sign (+, − or 0) for each residue is shown below the amino acid sequence. Secondary structural elements derived from the NMR chemical shift data are shown as boxes (helices) and arrows (−strands). Unassigned residues are marked by an asterisk. c) Secondary chemical shifts for ^{13}C are plotted as a function of residue number.

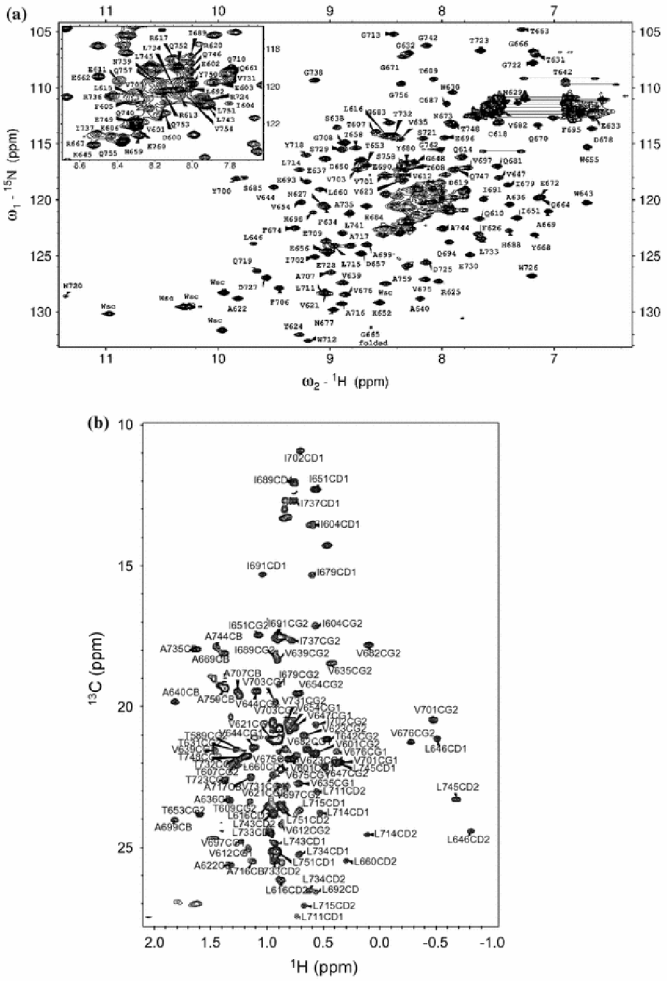

Figure 2.

Two-dimensional NMR spectra ^{1}H,^{15}N-HSQC (a) and ^{1}H,^{13}C-HSQC (b) of NpR6012g4 in the green-absorbing photoproduct recorded at 600 MHz proton frequency. Representative NMR assignments are indicated by the peak labels. The Asn and Gln sidechain amide peaks are connected with solid lines. The inset shows an expanded view of the heavily overlapped spectral region. Complete NMR assignments are available as BMRB accession no. 26577.