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¹H, ¹⁵N, and ¹³C chemical shift assignments of cyanobacteriochrome NpF2164g3 in the photoproduct state

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Abstract

Cyanobacteriochrome (CBCR) photosensory proteins are phytochrome relatives using bilin chromophores for light sensing across the visible spectrum. Structural information is not available for two of the four known CBCR subfamilies. NpF2164g3 is a member of one such subfamily, exhibiting a violet/orange photocycle. We report backbone NMR chemical shift assignments for the light-activated orange-absorbing state of NpF2164g3 (BMRB no. 19150).

Keywords

photoreceptor; CBCR; phytochrome; cyanobacteria; tetrapyrrole; NMR

Biological Context

Phytochromes are photosensory proteins utilizing covalently attached linear tetrapyrrole (bilin) chromophores. Light absorption triggers photoisomerization about the 15,16-bilin double bond, allowing the protein to photoconvert between red- and far-red-absorbing states (Auldridge and Forest 2011). In cyanobacteria, related cyanobacteriochrome (CBCR) sensors can detect red, green, blue, and even near-ultraviolet light (Ikeuchi and Ishizuka 2008; Rockwell, Martin et al. 2011). Phytochromes and CBCRs share a bilin-binding GAF domain with slight structural differences (Fig. 1A). For detection of blue to near-UV light, a second conserved Cys forms a covalent linkage to the bilin (Ishizuka, Kamiya et al. 2011; Rockwell, Martin et al. 2011). Such two-Cys photocycles evolved independently in the DXCF and insert-Cys CBCR subfamilies (Rockwell, Martin et al. 2011). There is no structural information for insert-Cys CBCRs, whose second Cys residue lies within an insertion loop in the CBCR GAF fold (Fig. 1A). The putative phototaxis sensor protein encoded by the *NpF2164* locus of *Nostoc punctiforme* contains six CBCR GAF domains in tandem. The third GAF domain is an insert-Cys CBCR called NpF2164g3.

NpF2164g3 switches between a dark-stable violet-absorbing state and a metastable orange-absorbing state (Rockwell, Martin et al. 2011). In the dark state, the phycocyanobilin (PCB) chromophore is covalently attached to the conserved CBCR cysteine (Cys591) at the bilin

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A-ring and to the insert-Cys cysteine (Cys546) at the bilin C10 atom. Photoconversion causes cleavage of the second thioether to form the orange-absorbing state. Biological signaling is thought to arise via propagation of these structural changes to adjacent domains, but atomic resolution structures of NpF2164g3 in both states are needed to elucidate such changes. We report detailed NMR resonance assignments for the light-activated state of NpF2164g3 (hereafter referred to as NpF2164g3) as a first step toward achieving this goal

Methods and Experiments

Expression and Purification of NpF2164g3

The protein sample in this study consists of 201 native residues with an N-terminal His-tag (MGSSHHHHHSSGLVPRGSHM). Expression and purification of NpF2164g3 were optimized for protein yield and chromophore incorporation. Best results were obtained using an N-terminally His-tagged construct with co-production of PCB in *E. coli* grown in commercial ^{15}N - or $^{15}\text{N}/^{13}\text{C}$ -labeled rich media (BioExpress, Cambridge Isotopes), followed by purification on Ni-NTA resin with elution using an imidazole gradient (Rockwell, Martin et al. 2012). Peak 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). Dark reversion of the metastable orange-absorbing state was 10% after 48 hours at 300 K, and both states were stable and active up to 318 K. For NMR characterization of the orange state, 1.2 mM deuterated *tris*-carboxyethyl phosphine (Cambridge Isotopes) was added to suppress oxidation of the insert-Cys Cysteine. The sample was illuminated with a 405 nm class IIIa laser pointer (LightVision Technologies) to photoequilibrium, with the orange-absorbing state present at 85%. Subsequent manipulations were performed in darkness. The protein was concentrated to 0.7 mM and D_2O was added to 7% (v/v).

NMR spectroscopy

NMR experiments were conducted using Bruker Advance 600 MHz spectrometer equipped with a triple resonance cryogenic probe. All experiments were performed in darkness, with spectral acquisition at 303 K. Backbone chemical shift assignments were obtained using ^1H , ^{15}N -HSQC, HNCA, HNCB, HNCACB, HNCACO, CBCACONH, HBHACONH, ^1H , ^{15}N -HSQC-TOCSY (mixing time of 60 ms), and ^1H , ^{15}N -NOESY-HSQC (mixing time of 120 ms) spectra (Ikura, Kay et al. 1990). NMR data were processed using NMRPipe (Delaglio, Grzesiek et al. 1995) software package and analyzed using SPARKY (www.cgl.ucsf.edu/home/sparky).

Assignments and Data Deposition

Figure 2 presents HSQC spectra of NpF2164g3 to illustrate representative backbone resonance assignments. NMR assignments were based on 3D heteronuclear NMR experiments performed on $^{13}\text{C}/^{15}\text{N}$ -labeled NpF2164g3 (residues 463–664). All non-proline residues exhibited strong backbone amide resonances with uniform intensities, indicative of a well-defined three-dimensional protein structure. More than 90% of the backbone resonances (^1HN , ^{15}N , $^{13}\text{C}\alpha$, $^{13}\text{C}\beta$, and ^{13}CO) were assigned. The chemical shift

assignments (^1H , ^{15}N , ^{13}C) of NpF2164g3 have been deposited in the BioMagResBank (<http://www.bmrb.wisc.edu>) under accession number 19150.

We used chemical shift index (Wishart, Sykes et al. 1992) to assign protein secondary structure for NpF2164g3 (Fig. 1B). This secondary structure is broadly similar to that of the closest relative of known structure, the CBCR AnPixJ (Narikawa, Ishizuka et al. 2013). However, there are four differences: (1) the kinked third beta strand of AnPixJ is apparently random coil in NpF2164g3; (2) the second of the pair of short helices before the fourth beta strand in AnPixJ is random coil in NpF2164g3; (3) the fourth helix in AnPixJ containing the conserved CBCR Cys is random coil in NpF2164g3; (4) the C-terminal helix of AnPixJ contains a short region of random coil in NpF2164g3. Using numbering based on AnPixJ, the secondary structure elements of NpF2164g3 are $\alpha 1$: 471–475; $\alpha 2$: 481–495; $\beta 1$: 499–506; $\beta 2$: 512–519; $\alpha 3A$: 559–565; $\beta 4$: 577–580; $\beta 5$: 602–611; $\beta 6$: 617–624; $\alpha 5A$: 632–642; and $\alpha 5B$: 647–660. The conserved CBCR Cys is at the N-terminal end of a helix in all phytochrome and CBCR structures reported to date, but that helix is absent in NpF2164g3 (see shaded box in Fig 1B), with four of those residues in random coil (residues 596–599) and the rest, including Cys591, unassigned. The characteristic insertion loop of the insert-Cys CBCRs (residues 523–559, highlighted blue in Fig. 1) exhibits random coil chemical shift index throughout, except for a short region including Cys546 (residues 541–546) that is not assigned. Future work on the NpF2164g3 dark state will allow us to determine whether these structural changes are general adaptations of the insert-Cys subfamily or if they are specific to the orange-absorbing photoproduct state.

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Fig. 1.

Primary sequence and secondary structure of NpF2164g3. (A) Topology diagrams for the CBCR AnPixJ in the red-absorbing dark state (left, (Narikawa, Ishizuka et al. 2013)) and NpF2164g3 in the orange-absorbing photoproduct (right, this work). (B) Detailed view of NpF2164g3. Secondary structural elements were derived from analysis of chemical shift index (Wishart, Sykes et al. 1992) and sequential NOE patterns. The chemical shift index sign (+, – or 0) is indicated underneath each residue. Residues in the insert-Cys insertion loop are highlighted blue. The two cysteine residues (Cys546 and Cys591) that form a dual Cys linkage to the dark-state chromophore are highlighted red. Unassigned residues are marked by an asterisk and by dashed lines in the structure cartoon. Residues in the shaded box region are helical in AnPixJ (Narikawa, Ishizuka et al. 2013).

Fig. 2. Two-dimensional ^1H , ^{15}N -HSQC NMR spectra of NpF2164g3 in the orange-absorbing form recorded at 600 MHz proton frequency. Side chain amide resonances of Asn and Gln are connected with solid lines. Representative assignments are indicated; complete assignments are available as BMRB accession no. 19150. The most downfield resonance (assigned to N624) is likely caused by a ring current effect. Unassigned peaks with weak intensity are resonances from the dark-state NpF2164g3, present at 15%.