

^1H , ^{13}C , and ^{15}N resonance assignment of photoactive yellow protein

Trijntje J. Pool · Nur Alia Oktaviani ·
Hironari Kamikubo · Mikio Kataoka ·
Frans A. A. Mulder

Received: 2 January 2012 / Accepted: 6 April 2012 / Published online: 19 April 2012
© The Author(s) 2012. This article is published with open access at Springerlink.com

Abstract Photoactive yellow protein (PYP) is involved in the negative phototactic response towards blue light of the bacterium *Halorhodospira halophila*. Here, we report nearly complete backbone and side chain ^1H , ^{13}C and ^{15}N resonance assignments at pH 5.8 and 20 °C of PYP in its electronic ground state.

Keywords PYP · *Halorhodospira halophila* · *para*-coumaric acid · NMR spectroscopy · Photoactivation

Biological context

Photoactive yellow protein (PYP) is a 125 amino acid (14 kDa) water-soluble, blue-light sensor protein, first found in the halophilic bacterium *Halorhodospira halophila* (Meyer 1985). PYP is a photoreceptor, believed to be responsible for the negative phototactic response of its host organism (Sprenger et al. 1993). This kind of response is required for organisms to evade potentially harmful short-

wavelength light. Based on this observation, PYP has become a suitable model to understand the signal-transduction mechanism in Per-Arnt-Sim (PAS) domain signaling (Crosthwaite et al. 1997; Nambu et al. 1991). Several PYP-like proteins have meanwhile been found in other organisms, where they are also thought to act as light sensors. In addition, PYP-like proteins found in purple bacteria are involved in cell buoyancy or sensing bacteriophytochromes (Jiang et al. 1999; Kyndt et al. 2004).

Understanding of light transduction in PYP requires structural information in atomic detail. A 1.4 Å crystallographic structure was determined in 1995 by Borgstahl et al. and in 1998 Düx and coworkers revealed the solution structure and backbone dynamics of PYP by NMR spectroscopy. The reaction center of PYP is protected from solvent by R52, which is believed to function as a gateway in the photocycle (Borgstahl et al. 1995; Genick et al. 1997). The chromophore, *para*-coumaric acid (*pCA*), is covalently bound to C69 with a thioester bond and *pCA* participates in two short hydrogen bonds with E46 and Y42 to stabilize the negative charge of *pCA* in the electronic ground state, pG. Upon blue-light capture, the chromophore undergoes *trans-cis* isomerisation and the intermediate pR is formed, which subsequently relaxes to the proposed signaling state, pB. In the latter state, the reaction center is exposed and the two short hydrogen bonds are broken (Borgstahl et al. 1995; Sigala et al. 2009; Yamaguchi et al. 2009).

In this paper, we present the nearly complete assignment of the backbone and side chain resonances of the pG state of PYP.

Methods and experiments

Uniformly ^{13}C , ^{15}N -labeled wild type PYP was overexpressed and purified as described previously (Düx et al.

T. J. Pool · N. A. Oktaviani · F. A. A. Mulder
Groningen Biomolecular Sciences and Biotechnology Institute,
University of Groningen, Nijenborgh 4, 9747 AG Groningen,
The Netherlands

H. Kamikubo · M. Kataoka
Graduate School of Materials Science, Nara Institute of Science
and Technology, 8916-5 Takayama, Ikoma, Nara 630-0192,
Japan

Present Address:

F. A. A. Mulder (✉)
Department of Chemistry, Interdisciplinary Nanoscience Center
(iNANO), University of Aarhus, Langelandsgade 140, 8000
Aarhus C, Denmark
e-mail: fmulder@chem.au.dk

1998). The NMR sample contained 1.2 mM doubly labeled [^{13}C , ^{15}N] PYP, 5 mM potassium phosphate buffer pH 5.8, 10 % D_2O and 0.15 mM 2,2-dimethyl-2-silapentane-5-sulfonic acid (DSS). All 1D ^1H , 2D and 3D NMR experiments were carried out at 20 °C using a Varian Unity INOVA 600 MHz spectrometer equipped with a field-gradient probe. Referencing was performed according to the method described by Wishart et al. (1995) using DSS. To obtain the sequential backbone resonance assignments, 2D ^{15}N -HSQC equipped with sensitivity enhancement and water flip back pulses (Kay et al. 1992; Zhang et al. 1994), 3D CBCA(CO)NH/HNCACB (Wittekind and Mueller 1993) and 3D HNC(O)HN(CA)CO (Ikura et al. 1990; Yamazaki et al. 1994) experiments were used.

To accomplish most of the side chain assignments, the strategy outlined by Oktaviani, et al. (2011) was performed. 3D (H)C(CO)NH-TOCSY and H(CCO)NH-TOCSY were used to assign carbon and proton aliphatic side chain resonances, respectively (Grzesiek et al. 1993; Logan et al. 1993; Montelione et al. 1992). For aromatic side chains, CB(CGCD)HD, CB(CGCDCE)HE (Yamazaki et al. 1993a), ^1H - ^{13}C HSQC CParo (Zuiderweg et al. 1996), 2D ^{13}C - ^1H CT HSQC, and 2D ^{13}C - ^1H CT HSQC_{ar} were recorded. Aromatic C^γ - H^β correlations were detected using CG(CB)HB experiments (Prompers et al. 1998). Carbonyl ^{13}C side chain resonances of Asx and Glx residues were assigned using H2(C)CO (Kay et al. 1990; Powers et al. 1991; Yamazaki et al. 1993b) and (HBGCBG)CO(CBGCABCON)H (Tollinger et al. 2002) experiments.

N^ζ - H^ϵ (Lys) and N^ϵ - H^δ (Arg) correlations were detected using H2(C)N (André et al. 2007).

All spectra were processed using NMRPipe (Delaglio et al. 1995) and they were analyzed with Sparky (Goddard and Kneller 2008).

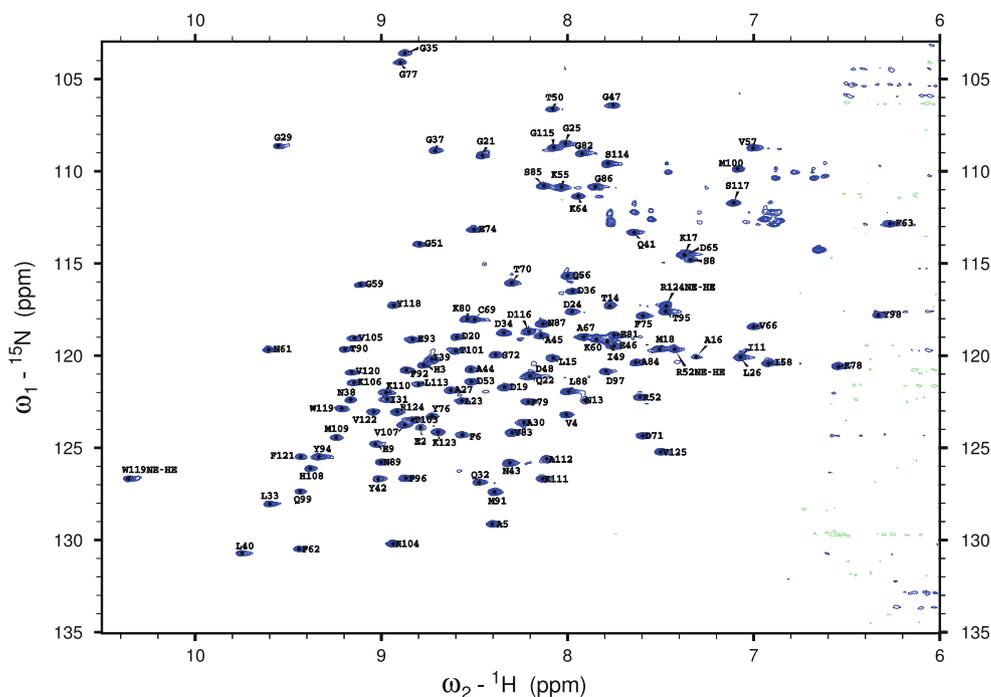
Completeness of assignments and data deposition

The obtained backbone assignment is over 96 % complete (see Fig. 1). Missing assignments are the amide backbone ^{15}N frequencies of the four Pro residues, and the amide nitrogen and proton frequencies of M1 and G7. At this pH no backbone assignment was possible for E12 and the $^{13}\text{C}'$ frequencies of F6, D10 and I11 were not found.

The entire ^1H , ^{13}C and ^{15}N side chain resonance assignment is over 85 % complete. Unassigned side chain resonances mainly pertain to labile protons and their attached heteroatoms, such as N^H/H^N of the two Arg residues, protons connected to oxygen (i.e. Tyr, Ser and Thr OH), protons bound to nitrogen and their corresponding nitrogens in the imidazole moiety of His residues, H^ζ of Lys, and also resonances due to several non-labile groups, such as methyl $\text{C}^\epsilon/\text{H}^\epsilon$ of Met residues, and a number of signals due to Phe and Trp ring systems.

The C^ζ frequency of the tyrosine residues were found in the ^1H - ^{13}C HSQC CParo experiment (run overnight) and could be assigned by using a combination of CB(CGCD)HD and CB(CGCDCE)HE spectra. However, Y76 and Y118

Fig. 1 2D ^{15}N -HSQC spectrum of PYP at pH 5.8 and 20 °C



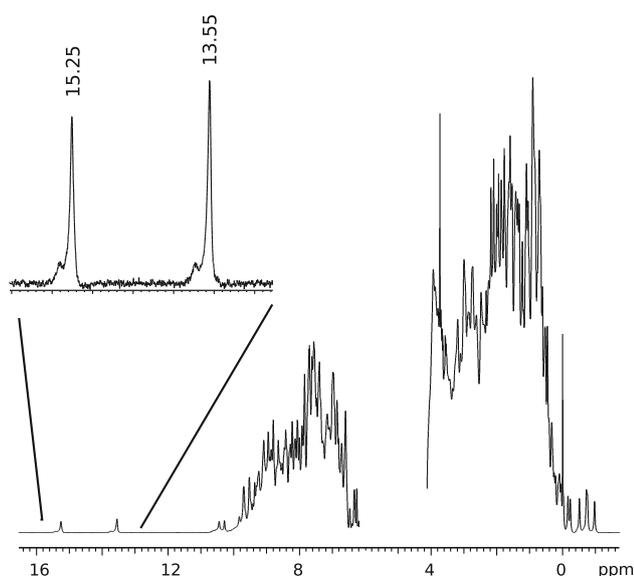


Fig. 2 1D ^1H NMR spectrum of PYP at pH 5.8, obtained with a selective water flip-back pulse, to detect the hydrogen bonds donated by Y42 and E46 to the chromophore oxygen. The downfield region is enlarged and the hydroxyl resonances of E46 and Y42 are observed at 15.25 and 13.55 ppm, respectively

have too similar H^ϵ frequencies to be able to determine which $\text{H}^\epsilon\text{-C}^\zeta$ peak corresponds to which residue. Therefore this information was obtained from pH-titration experiments, using the fact that tyrosine C^γ and C^ζ chemical shifts change simultaneously by large amounts during this titration. As the Y76 signals start moving at a lower pH than those of Y118, it was possible to assign the C^ζ nuclei in this fashion. We also can detect signals due to two short hydrogen bonds, which belong to Y42 and E46, using a 1D proton water flip-back sequence that suppresses the water signal without saturating it (see Fig. 2). These NMR signals have been assigned by Sigala et al. (2009).

Previously, ^1H and ^{15}N chemical shift assignments have been made at 37 °C by D \ddot{u} x et al. (1998), but these have not been submitted to the BMRB. Our results agree well with these, with a few exceptions. ^1H , ^{13}C and ^{15}N assignments for the N-terminal deletion variant $\Delta 25\text{-PYP}$ at 20 °C are available under BMRB accession number 6321 (Bernard et al. 2005), and these show significant differences as a result of the removal of part of the native protein structure.

The ^1H , ^{13}C and ^{15}N assignment can be found in the BioMagResBank under accession number 18122.

Acknowledgments This work was supported by a VIDI career development award to F.A.A.M from The Netherlands Organization for Scientific Research (NWO).

Conflict of interest The authors declare that they have no conflict of interest.

Open Access This article is distributed under the terms of the Creative Commons Attribution License which permits any use, distribution, and reproduction in any medium, provided the original author(s) and the source are credited.

References

- André I, Linse S, Mulder FAA (2007) Residue-specific pK_a determination of lysine and arginine side chains by indirect N-15 and C-13 NMR spectroscopy: application to apo calmodulin. *J Am Chem Soc* 129:15805–15813
- Bernard C, Houben K, Derix N, Marks D, van der Horst M, Hellingwerf K, Boelens R, Kaptein R, van Nuland N (2005) The solution structure of a transient photoreceptor intermediate: delta25 photoactive yellow protein. *Structure* 13:953–962
- Borgstahl GEO, Williams DR, Getzoff ED (1995) 1.4 angstrom structure of photoactive yellow protein, a cytosolic photoreceptor—unusual fold, active-site, and chromophore. *Biochemistry* 34:6278–6287
- Crosthwaite SK, Dunlap JC, Loros JJ (1997) *Neurospora wc-1* and *wc-2*: transcription, photoresponses, and the origins of circadian rhythmicity. *Science* 276:763–769
- Delaglio F, Grzesiek S, Vuister GW, Zhu G, Pfeifer J, Bax A (1995) Nmrpipe - a multidimensional spectral processing system based on Unix pipes. *J Biomol NMR* 6:277–293
- D \ddot{u} x P, Rubinstenn G, Vuister GW, Mulder FAA, Hard K, Hoff WD, Kroon AR, Crielaard W, Hellingwerf KJ, Kaptein R (1998) Solution structure and backbone dynamics of the photoactive yellow protein. *Biochemistry* 37:12689–12699
- Genick UK, Borgstahl GEO, Ng K, Ren Z, Pradervand C, Burke PM, Srajer V, Teng TY, Schildkamp W, Mcree DE, Moffat K, Getzoff ED (1997) Structure of a protein photocycle intermediate by millisecond time-resolved crystallography. *Science* 275:1471–1475
- Goddard TD, Kneller DG (2008) SPARKY 3. University of California, San Francisco
- Grzesiek S, Anglister J, Bax A (1993) Correlation of backbone amide and aliphatic side-chain resonances in $^{13}\text{C}/^{15}\text{N}$ -enriched proteins by isotropic mixing of ^{13}C magnetization. *J Magn Reson B* 101:114–119
- Ikura M, Kay LE, Bax A (1990) A novel approach for sequential assignment of H-1, C-13, and N-15 spectra of larger proteins—heteronuclear triple-resonance 3-dimensional NMR-spectroscopy—application to calmodulin. *Biochemistry* 29:4659–4667
- Jiang ZY, Swem LR, Rushing BG, Devanathan S, Tollin G, Bauer CE (1999) Bacterial photoreceptor with similarity to photoactive yellow protein and plant phytochromes. *Science* 285:406–409
- Kay LE, Ikura M, Tschudin R, Bax A (1990) Three-dimensional triple-resonance NMR spectroscopy of isotopically enriched proteins. *J Magn Reson* 89:496–514
- Kay LE, Keifer P, Saarinen T (1992) Pure absorption gradient enhanced heteronuclear single quantum correlation spectroscopy with improved sensitivity. *J Am Chem Soc* 114:10663–10665
- Kyndt JA, Meyer TE, Cusanovich MA (2004) Photoactive yellow protein, bacteriophytochrome, and sensory rhodopsin in purple phototrophic bacteria. *Photochem Photobiol Sci* 3:519–530
- Logan TM, Olejniczak ET, Xu RX, Fesik SW (1993) A general method for assigning NMR-spectra of denatured proteins using 3D HC(CO)NH-TOCSY triple resonance experiments. *J Biomol NMR* 3:225–231
- Meyer TE (1985) Isolation and characterization of soluble cytochromes, ferredoxins and other chromophoric proteins from the halophilic phototrophic bacterium *Ectothiorhodospira-Halophil-a*. *Biochim Biophys Acta* 806:175–183

- Montelione GT, Lyons BA, Emerson SD, Tashiro M (1992) An efficient triple resonance experiment using carbon-13 isotropic mixing for determining sequence-specific resonance assignments of isotopically-enriched proteins. *J Am Chem Soc* 114: 10974–10975
- Nambu JR, Lewis JO, Wharton KA, Crews ST (1991) The *Drosophila single-minded* gene encodes a helix-loop-helix protein that acts as a master regulator of CNS midline development. *Cell* 67:1157–1167
- Oktaviani NA, Otten R, Dijkstra K, Scheek RM, Thulin E, Akke M, Mulder FAA (2011) 100 % complete assignment of non-labile ^1H , ^{13}C , and ^{15}N signals for calcium-loaded Calbindin $\text{D}_{9\text{k}}$ P43G. *Biomol NMR Assign* 5:79–84
- Powers R, Gronenborn AM, Marius Clore G, Bax A (1991) Three-dimensional triple-resonance NMR of $^{13}\text{C}/^{15}\text{N}$ -enriched proteins using constant-time evolution. *J Magn Reson* 94:209–213
- Prompers JJ, Groenewegen A, Hilbers CW, Pepermans HAM (1998) Two-dimensional NMR experiments for the assignment of aromatic side chains in C-13-labeled proteins. *J Magn Reson* 130:68–75
- Sigala PA, Tsuchida MA, Herschlag D (2009) Hydrogen bond dynamics in the active site of photoactive yellow protein. *Proc Natl Acad Sci USA* 106:9232–9237
- Sprenger WW, Hoff WD, Armitage JP, Hellingwerf KJ (1993) The eubacterium *Ectothiorhodospira-Halophila* is negatively phototactic, with a wavelength dependence that fits the absorption-spectrum of the photoactive yellow protein. *J Bacteriol* 175:3096–3104
- Tollinger M, Forman-Kay JD, Kay LE (2002) Measurement of side-chain carboxyl pK_a values of glutamate and aspartate residues in an unfolded protein by multinuclear NMR spectroscopy. *J Am Chem Soc* 124:5714–5717
- Wishart DS, Bigam CG, Yao J, Abildgaard F, Dyson HJ, Oldfield E, Markley JL, Sykes BD (1995) H-1, C-13 and N-15 chemical-shift referencing in biomolecular NMR. *J Biomol NMR* 6:135–140
- Wittekind M, Mueller L (1993) HNCACB, a high-sensitivity 3D NMR experiment to correlate amide-proton and nitrogen resonances with the alpha-carbon and beta-carbon resonances in proteins. *J Magn Reson B* 101:201–205
- Yamaguchi S, Kamikubo H, Kurihara K, Kuroki R, Niimura N, Shimizu N, Yamazaki Y, Kataoka M (2009) Low-barrier hydrogen bond in photoactive yellow protein. *Proc Natl Acad Sci USA* 106:440–444
- Yamazaki T, Formankay JD, Kay LE (1993a) 2-dimensional NMR experiments for correlating C-13-beta and H-1-delta/epsilon chemical-shifts of aromatic residues in C-13-labeled proteins via scalar couplings. *J Am Chem Soc* 115:11054–11055
- Yamazaki T, Yoshida M, Nagayama K (1993b) Complete assignments of magnetic resonances of ribonuclease-H from *Escherichia-Coli* by double-resonance and triple-resonance 2D and 3D NMR spectroscopies. *Biochemistry* 32:5656–5669
- Yamazaki T, Lee W, Arrowsmith CH, Muhandiram DR, Kay LE (1994) A suite of triple-resonance NMR experiments for the backbone assignment of N-15, C-13, H-2 labeled proteins with high-sensitivity. *J Am Chem Soc* 116:11655–11666
- Zhang O, Kay LE, Olivier JP, Forman-Kay JD (1994) Backbone ^1H and ^{15}N resonance assignments of the N-terminal SH3 domain of drk in folded and unfolded states using enhanced-sensitivity pulsed field gradient NMR techniques. *J Biomol NMR* 4:845–858
- Zuiderweg ERP, Zeng L, Brutscher B, Morshauer RC (1996) Band-selective hetero- and homonuclear cross-polarization using trains of shaped pulses. *J Biomol NMR* 8:147–160