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Letter to the Editor: ^{1}H, ^{15}N and ^{13}C backbone and side-chain assignments of the rice phytochrome B PAS1 domain and backbone assignments of the PAS1-PAS2 domain

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Biological context

Phytochromes consist of a family of red/far-red light photoreceptors in plants that regulate photomorphogenic events ranging from seed germination and deetiolation, to the induction of flowering (Neff et al., 2000). Following absorption of red light, phytochromes translocate from the cytoplasm to the nucleus, and subsequently regulate gene expression through interactions with transcription factors such as basic-helix-loop-helix proteins (Quail, 2002). Phytochrome proteins possess two major structural domains, the N- and C- terminal domains (Quail, 1997). The N-terminal domain (~74 kDa) possesses a covalently attached linear tetrapyrrole chromophore (phytochromobilin) and is sufficient for light absorption and photoreversibility. The C-terminal domain (~55 kDa), which plays a role in phytochrome dimerization and downstream signaling, consists of two PAS (PER-ARNT-SIM) domains and one histidine kinase-like domain. The PAS domains are a family of sensor protein domains involved in signal transduction in a wide range of organisms (Taylor and Zhulin, 1999). With phytochromes, a loss of function missense mutation cluster was located within the PAS domains, suggesting that these domains are crucial for phytochrome signaling (Quail et al., 1995; Ni et al., 1998; Chen et al., 2003; Matsushita et al., 2003). In an effort to delineate the mechanism underlying photomorphogenesis in plants, it is important to investigate the three- dimensional structure and dynamic aspects of this domain. The assignment of the rice phytochrome B PAS domains presented here represents the first step toward the study of phytochrome signaling using NMR.

Methods and experiments

The rice phytochrome B (phyB) PAS1 and PAS1-PAS2 domains (residues 666-782 and 666-923, respectively) were cloned into the pET32c expression vector (Novagen) and over-produced in *E. coli* BL21 (DE3) RIL (Novagen) as thioredoxin and hexa-histidine fusion proteins. $^{13}C/^{15}N$- or ^{15}N-labeled protein was induced by the presence of 1 mM IPTG at 25 °C in M9 minimal medium containing $^{15}NH_4Cl$ and [U–^{13}C] glucose or unlabeled glucose, respectively. Cells obtained from M9 cultures were lysed by sonication. The lysate was centrifuged and the supernatant was loaded onto a Ni-NTA agarose resin (Qiagen). Proteins were eluted with imidazole in a stepwise manner. The sample fraction was then passed through a Superdex 26/60 75 pg gel filtration column (Amersham). Following removal of the fused tag by enterokinase (Novagen) was performed, Hi-trap Q anion-exchange (Amersham) and Superdex 26/60 75 pg columns were used to further purify the samples. The identity and integrity of the protein samples was confirmed by N-terminal sequencing, MALDI/TOF MS and SDS-PAGE.

All NMR measurements were carried out on a Bruker AV500 spectrometer equipped with a $^{1}H/^{13}C/^{15}N$ cryogenic probe and a DRX800 spectrometer

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equipped with a $^1H/^{13}C/^{15}N/^{31}P$ probe at 30 °C. The PAS1 and PAS1-PAS2 domains were dissolved in 90%/10% H_2O/D_2O containing 50 mM phosphate (pH 6.8), 20 mM KCl and 5 mM DTT at a protein concentration of 0.8 mM and 0.2 mM, respectively. For the PAS1 domain, the backbone and side-chain resonances were obtained from the following spectra: HNCACB, HN(CO)CACB, HN(CA)CO, HNCO, C(CO)NH, H(CCO)NH, 4D-HC(CO)NH, HCCH-TOCSY, ^{15}N-edited TOCSY-HSQC and 2D-NOESY experiments. For the sequential backbone assignments of the PAS1-PAS2 domain, HNCACB, HN(CO)CACB, HN(CA)CO and HNCO were recorded. The NMR data were processed using NMRPipe (Delaglio et al., 1995) and analyzed using SPARKY (T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco).

Extent of assignments and data deposition

Supplementary Figure 1a and 1b show the $^1H/^{15}N$ HSQC spectra of the phyB PAS1 and phyB PAS1-PAS2 domains, respectively. The phyB PAS1 domain consists of 124 residues including 7 N-terminal residues originating from the vector, while the phyB PAS1-PAS2 domain consists of 261 residues including 3 residues from the vector. For the backbone atoms of the PAS1 domain, 97.5, 97.5, 97.5, 97.3 and 98.4% of the ^1HN, ^{15}N, $^{13}C_\alpha$, $^{13}C_\beta$ and $^{13}C'$ resonance assignments were obtained, respectively. For the aliphatic and aromatic side-chain atoms of the PAS1 domain, 92.7% of the ^1H and ^{13}C resonance assignments were obtained. For the PAS1-PAS2 domain, 98.4, 98.4, 98.9, 99.2 and 98.9% of the ^1HN, ^{15}N, $^{13}C_\alpha$, $^{13}C_\beta$ and $^{13}C'$ backbone resonance assignments were obtained. The L695-T696, L698, V709, I713-F714, K740-Q748 and K750-I756 in the PAS1 domain, and the V709, I713, T743-Q748, K750-G751 and V755 in the PAS1-PAS2 domain showed minor peaks. The chemical shift assignments (^1H, ^{15}N, ^{13}C) of the rice phyB PAS1 and PAS1-PAS2 domains have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 6439 and 6440, respectively.

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