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Letter to the Editor: ^{1}H, ^{13}C and ^{15}N chemical shift assignments of the N-terminal PAS domain of mNPAS2

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Abbreviations: bHLH, basic Helix-Loop-Helix domain; BMAL1, Brain and Muscle ARNT-Like protein 1; mNPAS2, murine Neuronal PAS protein 2; PAS domain, Period-ARNT-Single minded-like domain.

Biological context

PAS domains mediate interactions among proteins involved in regulating a wide range of biological functions, including gene expression. The protein-binding capabilities of several PAS domains are modulated by small organic ligands, providing a direct mechanism to modulate biological pathways in response to external stimuli. Insight into the molecular basis of this regulation has been provided by PAS domain structures solved to date, several of which are PAS/ligand complexes (Pellequer et al., 1999; Crosson and Moffat, 2001).

Neuronal PAS protein 2 (NPAS2) is a PAS-containing mammalian transcription factor that has high sequence homology to Clock (King et al., 1997). Both NPAS2 and Clock are elements of the circadian clock apparatus which, through the formation of DNA-binding heterodimers with BMAL1, rhythmically activate the expression of a number of target genes including *Per1, Per2* and *Cry* (Reick et al., 2001).

NPAS2, Clock and BMAL1 are members of the bHLH/PAS family of transcription factors. Formation of heterodimeric complexes among members of this family is thought to be mediated by interactions of both the bHLH and PAS domains of the two components. To detail the mechanism of PAS-based dimerization among these proteins, we are structurally characterizing the N-terminal PAS domain of murine NPAS2 (mNPAS2 (78–240)). Here we report the chemical shifts of this fragment, which establish that this exhibits a well-folded PAS domain topology.

Methods and experiments

Uniformly ^{13}C, ^{15}N labeled mNPAS2 (78–240) was produced in *E. coli* BL21(DE3) cells grown in M9 minimal media with 1 g $^{15}NH_4Cl$, 3 g U-^{13}C-glucose and 10 ml 10× ^{15}N,^{13}C-labeled Bioexpress cell growth media per liter (Cambridge Isotope Laboratories). Protein was expressed as a His_6-fusion in a pHIS.Parallel1 vector (Sheffield et al., 1999) and purified by nickel affinity chromatography. This protein was digested with His-tagged TEV protease, followed by a nickel affinity purification step to remove the protease and cleaved His_6-tag. Approximately 20 mg/l of protein were obtained by this approach, the identity of which was confirmed by mass spectrometry (predicted MW = 18714.2 Da, actual MW = 18715.0 Da). Uniformly ^{2}H, ^{15}N-labeled samples were produced in a similar manner in M9 minimal media with 99% D_2O (Gardner and Kay, 1998) using 3 g/l d_8-glycerol as the carbon source in media used during protein induction. Approximately 20 mg/l protein were purified, with 99% deuteration of non-exchangeable sites as determined by mass spectrometry.

NMR samples contained 1 mM protein in 20 mM NaP_i, 20 mM NaCl, 5 mM DTT, 0.04% NaN_3, protease inhibitor cocktail (Sigma Aldrich, used at manufacturer's recommended concentration), 10% D_2O, pH = 7.5. Spectra were acquired on Varian 500 MHz and 600 MHz Inova spectrometers at 25 °C or 30 °C. Data was processed using NMRPipe (Delaglio et al., 1995) and analyzed with NMRView 4.0 (Johnson and Blevins, 1994).

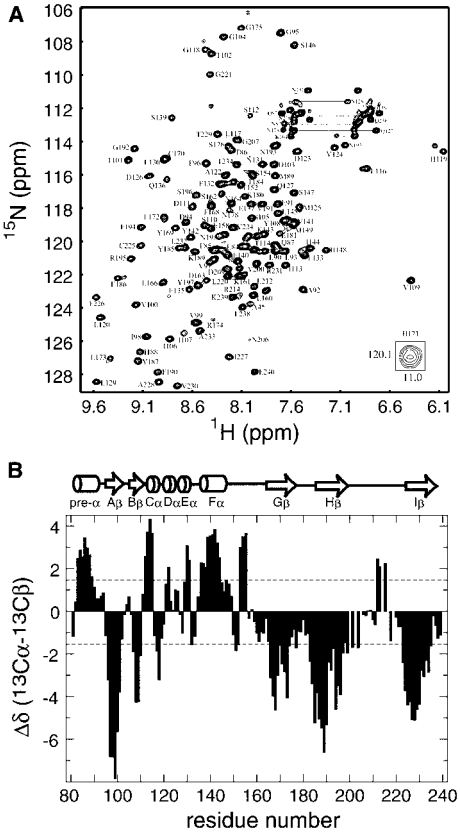

Figure 1. (A) ^{15}N/^{1}H HSQC spectrum of mNPAS2 (78–240). Vector-derived residues are labeled with asterisks and doubled peaks are connected by dashed lines. (B) Secondary structure elements of mNPAS2 (78–240) as determined by the Chemical Shift Index method. This index is calculated by calculating a three-point running average of combined C_α and C_β chemical shift indices ($\Delta\delta[^{13}C_\alpha]$-$\Delta\delta[^{13}C_\beta]$). Secondary shifts greater than $\pm$1.4 ppm (dashed lines) are considered to be significant.

Backbone assignments were obtained as shown in Figure 1A using CBCA(CO)NH, HNCACB, HNCO, (HCA)CO(CA)NH and HNHA data. Secondary structure assignments were obtained from C_α and C_β chemical shifts as shown in Figure 1B (Wishart and Sykes, 1994). Sidechain chemical shift assignments were hampered by poor signal-to-noise ratios in C(CO)NH TOCSY experiments recorded on ^{13}C,^{15}N protein as well as limited sample lifetimes (50% reduction in peak intensity over ~6 days at 25 °C). Tertiary structure information was obtained from two 3D ^{15}N-edited NOESY experiments (τ_m = 150 ms, 400 ms) recorded on uniformly ^{2}H, ^{15}N-labeled protein samples. Additional information was derived from deuterium exchange experiments of ^{15}N-labeled protein.

Extent of assignments and data deposition

Despite the modest stability of mNPAS2 (78–240), we were able to obtain backbone assignments for 93% of non-proline residues. Analyses of these chemical shifts establish that this domain has the mixed α/β topology typical of PAS domains (Pellequer et al., 1999). This was further confirmed by patterns of NOESY-derived NH-NH distances and NH protection in ^{2}H exchange experiments. mNPAS2 (78–240) is the largest structurally characterized member of the PAS family, chiefly due to the extension of several loops between strands in the central β sheet. Interestingly, several residues show peak doubling in the ^{15}N-^{1}H HSQC spectrum, suggestive of slowly-exchanging multiple conformations. Most of these residues are located in two loops near the region used for ligand binding in the PYP PAS domain (Pellequer et al., 1999). ^{1}H, ^{15}N, $^{13}C_\alpha$, $^{13}C_\beta$, ^{13}CO and $^{1}H_\alpha$ chemical shifts have been deposited in the BioMagResBank under accession number 5019.

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