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Letter to the Editor: Assignment of the ^{1}H, ^{13}C, and ^{15}N resonances of the AXH domain of the transcription factor HBP1

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Biological context

A small motif (ca. 130 amino acids long), named AXH module, has recently been identified in HBP1 and in the apparently unrelated protein ataxin-1 (ATX1) (Mushegian et al., 1997). HBP1 is an ubiquitous transcription factor thought to be involved in an architectural regulation of chromatin and in specific gene expression (Zhuma et al., 1999). HBP1 is known to interact with retinoblastoma (RB) proteins, a family of transcriptional co-factors involved in cellular differentiation, tissue-specific gene expression and apoptosis protection (Yee et al., 1998). ATX1 is responsible for the spinocerebellar ataxia type-1 (SCA1), an autosomal-dominant neurodegenerative disorder characterised by ataxia and progressive motor deterioration and caused by expansion of unstable CAG trinucleotide repeats in the coding region of the corresponding gene (Zoghbi and Orr, 1995). In SCA1 patients, ATX1 forms intranuclear inclusions which lead to neuronal death. ATX1 is known to interact with RNA as well as with several protein partners, such as the proteins LANP, USP7 and p80 coilin (de Chiara et al., 2003). The AXH motif is differently located within the sequences of HBP1 and ATX1, whose architectures are anyway very different (Mushegian et al., 1997). The motif spans residues 570–689 of ATX1 (the numbering is refereed to human ATX1), whereas in HBP1 it is located approximately in the middle of the protein (210–340 of the human sequence) and followed by a C-terminal HMG group.

We have recently shown that the AXH modules of both HBP1 and ATX1 fold into thermodynamically stable globular domains (de Chiara et al., 2003). Identification of the AXH domain boundaries by comparative biophysical studies allowed us to map several sites of interaction with other molecules onto the AXH module, thus suggesting that AXH is a novel protein-protein and RNA binding motif. Structure determination of the domain is the next logical step to provide new insights into the molecular determinants of these interactions and a more general understanding of the cellular role of the AXH domain.

Here we report an essentially complete backbone and side-chain assignment for the AXH domain of HBP1.

Methods and experiments

The AXH domain of the *M. musculus* HBP1 (residues 208–345) was produced as a six-His tag glutathione transferase (GST) fusion protein with a recombinant tobacco endovirus (rTEV) protease recognition site resulting in four non-native residues (GAMA) at N-terminus of the protein. Isotopically ^{15}N-labeled and ^{13}C/^{15}N double labeled samples were over-expressed in *E. coli* host strain BL21 (DE3) grown on a minimal media containing 1 g l^{-1} ^{15}N-ammonium sulphate and 2 g l^{-1} ^{13}C-glucose and purified as previously described (de Chiara et al., 2003). The NMR samples concentration was 0.7 mM, in a buffer containing 20 mM Tris-HCl, 10 mM NaCl and 2 mM β-mercaptoethanol at pH 7.0 either in 90% H_2O/10%D_2O or in 99% D_2O. β-mercaptoethanol was always used to avoid oxidation of the seven cysteine residues. All NMR experiments were acquired at 303 K on Varian Inova spectrometers operating at 14.1 and 18.8 T.

HNCA, HN(CO)CA, HNCO, HNCACB experiments were employed to obtain sequence specific ^{1}HN, ^{15}N, ^{13}Cα, ^{13}Cβ and ^{13}C′ backbone assignments (Muhandiram and Kay, 1994). Side chain aliphatic proton and carbon assignments were achieved by a combination of 3D ^{15}N-edited TOCSY- and NOESY-HSQC (Fesik and Zuiderweg, 1988), HNHA, HNHB, C(CO)NH, H(CCO)NH and HCCH-TOCSY (Kay et al., 1993). The ^{1}H and ^{13}C assignment of P336, followed by another proline residue, was initially

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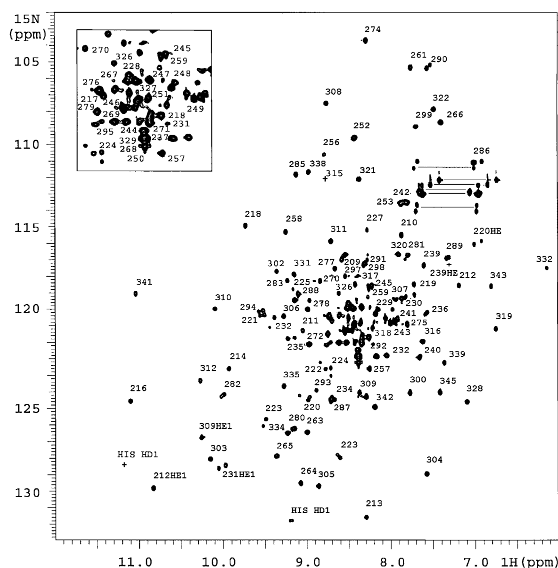

Figure 1. 2D ^{1}H, ^{15}N-HSQC spectrum of the mM HBP1 AXH domain. The NH_2 side chain resonances are connected by continuous lines.

achieved by looking for the expected sequential NOEs in the ^{13}C-edited NOESY and then completed by ^{13}C-HSQC and HCCH-TOCSY. For the assignment of the large number (22) of aromatic side chains present in HBP1 AXH, (Hβ)Cβ(CγCδ)Hδ and (Hβ)Cβ (CγCδ)Hε experiments were used in combination with ^{13}C-HSQC and HCCH-TOCSY tuned for the aromatic resonances.

All spectra were processed using NMRPipe/NMR-Draw (Delaglio et al., 1995) and analysed using XEASY (Bartels et al., 1995)

Extent of assignment and data deposition

The HBP1 AXH domain resulted in NMR spectra of excellent quality. In the ^{15}N-HSQC (Figure 1), backbone NH resonances were observed for 129 out of an expected 130 non-proline residues.

The NMR assignment of ^{1}H, ^{13}C and ^{15}N backbone resonances is complete for all but one residue with the exception of the C′ atoms of the residues followed by prolines. Only H254 remains completely unassigned. The assignment of side chain aliphatic ^{1}H and ^{13}C resonances, $^{15}NH_2$ of asparagine and glutamine residues and the NεHε and guanidinium groups of arginines is complete. Aromatic proton and carbon assignment is approximately 92% complete, a minor number remaining unassigned owing to missing correlations and/or resonance overlap. The resonances of residues G256, F273, and S315 are very weak possibly because of internal mobility of these residues on a slow or intermediate timescale. It was possible to assign the hydroxyl proton of T219. Two HD1 of histidine protons are unusually protected from solvent-exchange and are observed in the ^{1}H-^{15}N-HSQC spectrum (Figure 1), appearing at $\delta(^{1}H) = 9.19, 11.20$ ppm aliased from their true chemical shift at $\delta(^{15}N) = 165.7, 162.7$ ppm, respectively (^{15}N spectral width 32.99 ppm). However, it is not possible at this stage to assign them unambiguously to one of the eight histidines.

As an effect of the aging of the sample, a secondary species would appear with time for about 15% of the residues. The aminoacids involved have all been identified and show a similar pattern of NOEs in the two forms. Since most of them cluster around cysteine residues, a possible explanation is the covalent linking of β-mercaptoethanol molecules to some solvent exposed cysteines. This hypothesis, rather than cysteine-promoted intermolecular dimerization, is supported by electrospray mass spectrometry as well as by sodium dodecyl sulphate (SDS) page analysis (under oxidant conditions).

The of ^{1}H, ^{13}C and ^{15}N chemical shifts of the AXH domain of HBP1 have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number BMRB-5884.

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References

Bartels, C., Xia, T., Billiter, N., Güntert, P. and Wüthrich, K. (1995) *J. Biomol. NMR*, **6**, 1–10.

de Chiara, C., Giannini, C., Adinolfi, S., de Boer, J., Guida, S., Ramos, A., Jodice, C., Kioussis, D. and Pastore, A. (2003) *FEBS Lett.*, **551**, 107–112.

Delaglio, F., Grzesiek, S., Vuister, G., Zhu, G., Pfeifer, J. and Bax, A. (1995) *J. Biomol. NMR*, **6**, 277–293.

Fesik, S.W. and Zuiderweg, E.R.P. (1988) *J. Magn. Reson.*, **78**, 588–593.

Kay, L.E., Xu, G., Singer, A.U., Muhandiram, D.R. and Forman-Kay, J.D. (1993) *J. Magn. Reson.*, **B101**, 203–216.

Muhandiram, D.R. and Kay, L.E. (1994) *J. Magn. Reson.*, **B103**, 203–216.

Mushegian, A.R., Bassett, D.E. Jr, Boguski, M.S., Bork, P. and Koonin, E.V. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 5831–5836.

Yee, A.S, Shih, H.H. and Tevosian, S.G. (1998) *Front. Biosci.*, **3**, D532–547.

Zhuma, T., Tyrrell, R., Sekkali, B., Skavdis, G., Saveliev, A., Tolaini, M., Roderick, K., Norton, T., Smerdon, S., Sedgwick, S., Festenstein, R. and Kioussis, D. (1999) *EMBO J.* **18**, 6396–6406.

Zoghbi, H.Y. and Orr, H.T. (1995) *Semin. Cell Biol.*, **6**, 29–35.