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Letter to the Editor: ^{1}H, ^{13}C, and ^{15}N resonance assignments of the hepatocyte nuclear factor 6α (HNF-6α)

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Biological context

HNF-6 is a transcription factor identified in many tissues and specially enriched in liver (Lemaigre et al., 1996; Rausa et al., 1997). The DNA recognition of HNF-6 involves a cut domain and a homeodomain, but the latter is dispensable for binding to a subset of sites (Lannoy et al., 1998). Cut homeodomain proteins were originally described as the products of *Drosophila* cut gene and of its mammalian homologues the mclox genes (Harada et al., 1994), and these proteins contain three cut domains upstream of a homeodomain. The cut domains are constituted from 70 to 80 conserved residues, and function as DNA binding domains alone or in combination with a homeodomain. HNF-6 binds to a family of homologous DNA binding sites. The DNA binding domains of HNF-6 are regulated by these DNA sequences to recruit different cofactors in transcription regulation (Lannoy et al., 2000). A recent experiment further demonstrated that the cut domain and homeodomain of HNF-6 interacted with the winged helix DNA binding domain of FoxA2 (also called HNF-3β) both *in vivo* and *in vitro*, and their interaction blocked HNF-6 binding to DNA, but synergized FoxA2 activity on a FoxA2 binding site (Rausa et al., 2003). Apparently, detailed studies of the structure of the cut and homeo domains of HNF-6 and their interactions with DNA and proteins will reveal the versatile mechanisms used by the cut and homeo domains of HNF-6 in transcription regulation. HNF-6 has two forms originating from alternative slicing. Both 6α and 6β contain the identical cut domain and homeodomain. HNF-6β has an extra 26 amino acid residues inserted in the linker of HNF-6α, and the two forms show slightly different DNA binding properties (Lannoy et al., 2000). As the first step, here we report backbone and side-chain ^{1}H, ^{13}C and ^{15}N chemical shift assignment for the HNF-6α from mouse. The chemical shift values are critical to our study on how the cut and homeo domains can recognize the divergent protein and DNA targets.

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Methods and experiments

Expression and purification of HNF-6α

The gene encoding the DNA binding domains (solely referred as HNF-6 from now on) of HNF-6 protein was a generous gift from Dr. Robert Costa at University of Illinois at Chicago. Expression and purification of uniformly labeled HNF-6α is carried out by a standard procedure using Ni-NTA resin under denaturing conditions [Qiagen Manual (1999), 3rd edn.]. Purified HNF-6 was renatured by dialysis against a phosphate buffer (50 mM Na_2HPO_4, PH 6.5 and 50 mM NaCl). At the end of dialysis, a mixture of protease inhibitors (Sigma) is present to re-nature HNF-6 solution in about 1 to 20 dilution. The renatured protein shows the expected molecular weight on a SDS gel and binds to its binding sites judged by gel-shift essays. In the NMR sample, 250 to 400 mM of urea was added in the different NMR samples. In this study, the urea in the NMR samples suppresses strong resonance signals from flexible residues in HNF-6 and facilitates the assignments of overlapped resonances by following urea concentration-dependent chemical shift changes of the residues in the unstructured sequences. Interestingly, the urea only has the minimum effect on the residues in the structured sequences. Finally, protein concentrations were in the range of 1 to 2 mM as measured by the BioRad protein assay using bovine serum albumin as the standard (BioRad Laboratories, Hercules, CA).

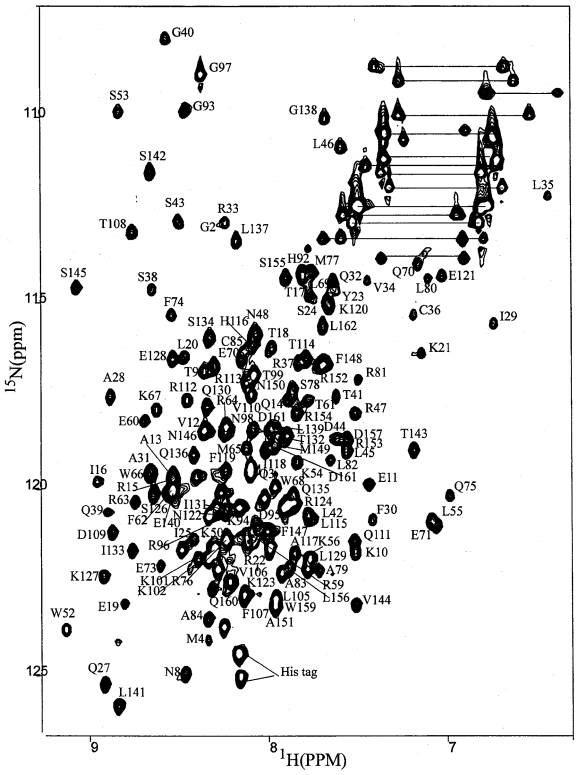

Figure 1. 2D ^{1}H-^{15}N HSQC spectrum at 600 MHz of HNF-6α obtained at 22 °C and pH 6.5 with 250 mM Urea. Backbone amide resonances are labeled with the one letter amino acid code and residue number. Peaks connected by solid line are from side-chain, unassigned.

NMR spectroscopy

NMR experiments were performed in 10%D_2O/90% H_2O or in 100% D_2O at 22 °C on Bruker DRX600, Varian INOVA 600 and 800 MHz spectrometers. The sequence-specific backbone assignments of 162 amino acid residues of HNF-6 were achieved through a combination of HNCA, HNCACB, CBCA(CO)NH, HNCO, and HN(CA)CO spectra, together with sequential NOES in NOESY-^{15}N-HSQC (120 ms on ^{15}N enriched protein and 160 ms on ^{15}N, ^{2}H enriched protein) spectra. Aliphatic side-chain chemical assignments were made using HNCACB, C(CO)NH, H(C)(CO)NH, and TOCSY-^{15}N-HSQC spectra, together with NOESY-^{13}C-HSQC (120 ms) spectrum. Aromatic ring proton and carbon resonances were assigned by analyzing 2D ^{1}H-^{13}C HSQC, ^{1}H-^{1}H TOCSY, and NOESY-^{13}C-HSQC (120 ms) spectrum. Furthermore, the side-chain assignments, such as γ^{m} of Ile and H^{δ} of Tyr and Phe, were facilitated by analyzing the side-chain to backbone NOEs observed in the ^{15}N, ^{2}H enriched protein, which has selective side-chain proton enrichment (Rosen et al., 1996). Data were processed and analyzed using commercial software SYBYL (Tripos Inc., MO). All ^{1}H dimensions were referenced to internal 2,2-dimethyl-2-silapentane-5-sulfonate (DSS), and ^{13}C and ^{15}N were indirectly referenced to DSS (Wishart et al., 1995).

Extent of assignments and data deposition

The ^{1}H-^{15}N HSQC spectrum of HNF-6α at 22 °C is shown in Figure 1. We have assigned approximately 93% of all possible resonances of backbone nuclei ($^{1}H^{N}$, ^{15}N, $^{13}C^{\alpha}$, $^{13}C^{\beta}$, $^{13}C'$, and $^{1}H^{\alpha}$) for HNF-6α. Assignments could not be made for residues Glu5, Lys56, Ser57, and several residues in the cut-homeo domain linker sequence (residues 86–91) due to the unresolvable overlaps and/or weak signals. The resonance of Ile6 in the cut domain is unusually $^{1}H^{N}$ upfield shifted (5.75 ppm), which is not displayed in Figure 1. It packs against aromatic residue Trp52 as implicated in the structural calculation. In addition, approximately 85% of the ^{1}H and ^{13}C resonances of side chains have also been assigned. This includes 10 of 14 aromatic residues. The assignments are available as supplementary material and have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 5678.

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